QUARTERLY JOURNAL

OF

MICROSCOPICAL SCIENCE:

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VOLUME XXV.—New Series.

With Lithographic Plates and Engrabings on Wood.

LONDON:

J & A. CHURCHILL, 11, NEW BURLINGTON STREET.

1885.

CONTENTS.

CONTENTS OF No. XCVIII, N.S., APRIL, 1885.

MEMOIRS:

CONTENTS OF No. XCIX, N.S., JULY, 1885.

MEMOIRS:

CONTENTS OF No. C, N.S., OCTOBER, 1885.

MEMOIRS:

SUPPLEMENT, 1885.

MEMOIRS:

The Significance of Kupffer's Vesicle, with Remarks on other Questions of Vertebrate Morphology.

By

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With Plate I.

SOME time ago I undertook the systematic study of the development of Teleosteans, because it seemed one of the more obscure departments of Vertebrate embryology. I was led to devote much attention to the herring, for of marine Teleosteans none offers more facilities to the embryologist.

The researches on which the following discussion is based have been carried on since the beginning of last August. My first acquaintance with herrings' eggs was made long before that time, namely in August, 1883, when I accompanied some members of the Scottish Fishery Board on an expedition to study the herring question in the Moray Firth. In the spring of the present year also, I obtained and artificially fertilized herring ova at the mouth of the Firth of Forth. But on neither of these occasions had I sufficient opportunities to make a study of the subject at all complete. In August of the present year I stayed about five weeks at the village of North Sunderland, on the Northumbrian coast, and there I was able to keep numbers of herring embryos in a healthy condition from fertilization up to the time of hatching and for ten days after. During the period of their development I

carefully examined as many successive stages in the living
condition as time would allow, and preserved specimens at
frequent intervals. These preserved examples I have since
studied by means of section-cutting in the laboratory of the
Scottish Marine Station.

My embryos were preserved by two methods. Some were
placed fresh in a saturated solution of corrosive sublimate,
others in weak solutions of chromic acid, generally about
$\frac{1}{5}$ per cent. In either case spirit was substituted after a
short time for the fixing solution. Neither of these methods
is perfectly satisfactory; the corrosive sublimate leaves the
embryos in too soft a condition, so that it is difficult to extract
them from the vitelline envelope and put them through the
processes of staining and embedding, without breaking them.
On the other hand, when sections are successfully cut they are
found to be satisfactorily stained and to show well the relations
of the layers. The embryos treated with chromic acid are very
hard and easy to manipulate, with the exception that the yolk
in cutting often breaks the other parts; but these embryos are
very refractory towards staining fluids, and the colouring of
the sections is never as differential as one would wish. Never-
theless, although my sections are not quite perfect in all
respects I have been able to make out a good deal as to the
development of the various organs. The following account
refers chiefly to the origin of the intestine, but I hope to clear
up some other points later. It is scarcely necessary to add
that my sections were cut with the microtome of Jung, by
means of the method perfected by Giesbrecht.

It is well known that the rate of development in herrings'
ova depends largely on temperature. I shall not here discuss
the various experimental data of this part of the subject, but
merely state the temperature to which my specimens were
exposed, and the period at which the chief stages were reached.
The first eggs I obtained were fertilized at 4.30 a.m., August
14th; the temperature of the surface of the sea at the time
was 13·3° C.; the temperature of the water where the eggs
were kept varied from 11·5° C. to 14·5° C. These eggs began

to hatch out on August 21st, the eighth day, and continued hatching on the following day. I next obtained eggs at 4 a.m., on August 26th; the temperature was about the same as before, and hatching took place on the eighth day.

As will be seen from fig. 1, Kupffer's vesicle is seen on the third day in the herring, by which time the eyes have appeared and the two ends of the embryo almost meet at the ventral pole of the yolk.

Historical.—Kupffer's vesicle was originally discovered and described by Kupffer[1] in 1868, in the embryos of Gasterosteus aculeatus, Gobius minutus, and Gobius niger. He named it "the allantois" without considering very carefully whether its relations corresponded to those of the allantois in the Amniota. In subsequent papers he has come to the conclusion that the vesicle is closely connected with the formation of the urinary ducts. Balfour in his ' Comparative Embryology,' states that Kupffer's vesicle is the representative of the post-anal vesicle in Elasmobranchs, without discussing the reasons which led him to this view. Kingsley and Conn[2] in 1883 described the origin of Kupffer's vesicle in Ctenolabrus. Their figures and descriptions are scanty, and refer only to optical sections. It is stated to arise from a number of small granules which fuse together; it is probable that these granules are small spaces between the periblast and hypoblast, and it makes no difference to my account of the vesicle whether it arises as a single space or from a number.

In a very recent paper by A. Agassiz and C. C. Whitman,[3] the following remarks are made concerning Kupffer's vesicle : " Although we have been able to trace the entire history of Kupffer's vesicle in several species of ova, its significance remains as complete a puzzle as ever. Kingsley and Conn were the first to give an accurate account of the origin of this vesicle,[4] but they give us no information in regard to its

[1] ' Arch. f. mik. Anat.,' Bd. iv.
[2] 'Memoirs of Boston Soc. of Nat. Hist.'
[3] 'Proc. Amer. Acad. of Arts and Sciences,' vol. xx, Aug., 1884.
[4] This remark is not altogether impartial—the vesicle is not correctly

subsequent history, and almost no details of its origin and growth. As they have stated the vesicle arises by the fusion or confluence of a cluster of granules. Those granules are at first few in number (2—4) more or less angular, quite dark, and not more than ·002 mm. in diameter. In general appearance they are not distinguishable from the scattered granules seen in other parts of the ovum. In Ctenolabrus they appear soon after the embryonic rim passes the equator. They increase in number, grow larger, coalesce by degrees, and finally blend into a single bubble-like vesicle in the course of five hours. This vesicle, ·01 mm. in diameter or more, more than doubles its diameter in the next hour and a half, and steadily expanding attains its maximum dimensions at the time the blastopore closes. During all this time it lies beneath the chorda and the entodermic stratum and has no sort of relation with any tubular structure whatever. As the alimentary canal is not yet in existence it is difficult to see how this vesicle can be the homologue of a dilatation which arises in and has no sort of existence outside of the post-anal gut. Ventrally and laterally it is bounded by periblastic material, but it has no cellular envelope in the strict sense of the words."

The authors then go on to describe the final history of the vesicle. Their description is not quite clear, but the meaning seems to be that the hypoblast above the vesicle is hollowed out to form a longitudinal furrow which deepens until a closed canal, the lumen of the gut, is formed, the depression in the periblast disappearing in the process. The authors think it probable that there exists from this time onward a lumen in the portion of the gut thus formed.

Agassiz and Whitman give no figures of the vesicle nor do they state if they confirmed their results by the examination of sections. Their paper has for its chief object to announce the important discovery that the nuclei of the periblast are origi-

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described when it is said to originate from globules, and Kingsley and Conn do not even point out its relation to the germinal layers.

nally derived by karyokinetic division from the nuclei of the cells at the edge of the blastoderm. Thus another supposed case of autogenous origin of nuclei falls to the ground.

I have given the exact relations of the vesicle at the time of its full development in the herring, as shown by a series of sections, in fig. 3. Figs. 2 and 4 show the relations of the layers behind and in front of the vesicle. It will be seen that at the period when the vesicle exists, the hypoblast is not distinctly differentiated from the mesoblast, and is not columnar. There is no distinct separation between the notochord and hypoblast, but the mesoblast is very sharply marked off from the notochord on each side. The two layers of the epiblast are distinctly seen, and the lower one is continuous with the neurochord. In the latter there is no central cavity; this appears later as seen in the subsequent figures. Both in front of the vesicle and behind it the hypoblast and periblast are in contact. The mesoblast does not extend far to the sides of the embryo; over the lateral and ventral parts of the yolk the periblast and epiblast are in contact.

The ventral wall of the gut in Elasmobranchs is formed by the differentiation of cells round the yolk-nuclei; in Amphibians by the differentiation of the superficial layer of the yolk-cells. There is little doubt that the floor of the gut in the herring is formed in an exactly similar way. As is shown by fig. 5, from an embryo sixty-four hours old, no periblast, or scarcely any, exists below the floor of the intestine, which is in this region complete: it is certain that no periblast nuclei are present here though they are seen beneath the lateral mesoblast and up to the side of the intestine. The same thing is shown by fig. 6, which is a transverse section passing through the otocysts. We may conclude, then, that this portion of the periblast has been used up to supply the cells of the floor of the gut. In the stage represented, figs. 5, 6, and 7, the intestine was not formed in the most anterior region of the body, and here, in front of the notochord, the neurochord comes into contact with the hypoblast of which there is a thin layer between the neural tissue and the periblast. This is shown in

the diagram fig. 8, and is referred to in a subsequent part of this paper.

In the stage represented in figs. 5, 6, and 7, the canal of the neurochord has appeared. In the posterior part of the embryo it is deep within the cord; in the anterior part it opens out above into a flat cavity covered only by a thin layer of epiblast. I was not able to satisfy myself whether this layer was only one cell deep, but it seemed to be so both above the neurochord and over the otocysts : it seems as if the lower layer went to form the neurochord and the wall of the auditory vesicle.

The explanation of Kupffer's vesicle in which my reflection on the subject has resulted is a very simple one.

In the Elasmobranch the invagination of the hypoblast which takes place at the posterior end of the blastoderm forms a tubular cavity open to the exterior by the blastopore. The dorsal wall of this cavity is formed by columnar hypoblast; its floor is occupied by the superficial layer of the yolk containing yolk-nuclei, that is by the periblast. This cavity n the Elasmobranch becomes shut off from the exterior by the closing of the medullary groove over the blastopore. In this way the cavity comes to be continuous posteriorly with the canal of the neurochord, a neurenteric canal being constituted which subsequently disappears. The history of this stage n the Amphibian is precisely similar, with the exception that in the Amphibian the segmentation is complete, and the yolk is wholly composed of large nucleated cells instead of containing nuclei only near its surface. The cavity of invagination of which I am speaking never obliterates, it becomes later the lumen of the intestine. The intestine is formed simply by the differentiation of the walls of the cavity. The dorsal wall of hypoblast is hollowed out longitudinally and its lateral parts approach one another; the floor of the alimentary canal is formed by cells differentiated round the nuclei of the periblast.

Kupffer's vesicle in the Teleostean is the homologue of the cavity of invagination in the Elasmobranch and Amphibians: it is the rudiment of the primitive gastrula-cavity, of that part

of it which is not represented by the body cavity. In the Teleostean it is never open to the exterior, but this need not surprise us since the cavity of the otocyst, or of the crystalline lens, or of the neurochord, is never open to the exterior in the Teleostean ; it is very doubtful also whether the neurenteric canal ever contains a lumen in Teleosteans. The intestine of the herring is, I believe, formed in exactly the same way as the intestine of the Elasmobranch or Amphibian from the gastrula-cavity ; fig. 5 shows that the floor of the intestine has been formed from the periblast.

It is of importance as favouring my view that in many Teleosteans Kupffer's vesicle is visible at a much earlier stage than the one in which I have seen it in the herring. In Gasterosteus aculeatus as described in Kupffer's paper of 1868, it is present when the blastoderm has got little beyond the equator of the yolk. The same is the case, as has been seen from the description of Agassiz and Whitman, in Ctenolabrus and probably in many other cases. This brings the period of the existence of the vesicle very closely into agreement with that of the existence of the gastrula cavity in Elasmobranchs. I am unable to say whether there is a neurenteric canal or anything representing it in the herring, which comes into relation with Kupffer's vesicle. On my view one would expect such a relation, but I must test this in the future.

I do not know whether to rejoice or regret that only in copying out this paper for the press I have found that a paper appeared in 1880, by M. Henneguy,[1] which advocates very much the same view as I now put forward. I have not yet referred to the paper and therefore do not know if M. Henneguy cut any sections. I found a few words which I had missed in the paper by Kingsley and Conn, stating that Henneguy believed he had found traces in the perch of an opening of invagination leading to the vesicle, and homologized the cavity with the primitive intestine in Cyclostomi and Batrachia, and the opening with the anus of Rusconi. Agassiz and Whitman

[1] 'Annals and Mag. Nat. Hist.,' ser. v, vol. vi.

simply say that the interpretations of Kupffer and Henneguy
are still more unsatisfactory than Balfour's and need not be
considered. I hope I have shown in this paper that the view
advocated by Henneguy and myself is the one which gives the
true morphological meaning of Kupffer's vesicle, and places it
on the same basis as the cavity of the canal of the neurochord
and of the otocyst in the Teleostean.

I have arrived at this view quite independently, and flattered
myself I was the first exponent of it. I find M. Henneguy has
anticipated me by four years. I hope my support of the view
will help to gain it the acceptance which so great an authority
as Agassiz has denied it.

There is one more question to be considered. Has the vesicle
any physiological importance in the process of actual develop-
ment? I think not; the intestine is formed in the anterior
part of the body without the aid of such a cavity, and I think
it is a true rudimentary structure which has persisted in spite
of the modifications in the development of the Teleostean
ovum, by reason of the strong tendency to perpetuate itself of
a structure so fundamental in the primitive stages of evolution
as the gastrula-cavity.

Remarks on General Vertebrate Morphology.

Ever since I became acquainted with the theory which
regards the Vertebrate as a worm turned on its back I have
been more and more convinced of its fundamental truth. Of
this theory Dohrn has been for years the most brilliant and
most profound exponent and investigator, and although he has
had, and may have again, occasionally to retrace his steps, he
has won for us a point of vantage from which we may look
back with clear view on the historical evolution of the Verte-
brate organization. Mr. Sedgwick and his school have not
embraced this theory; yet one point in Mr. Sedgwick's paper
published in this Journal, January, 1884, will, in my opinion,
do a very great deal towards completing the Dohrnian hypo-
thesis. The point I refer to is the stress laid on the fact that

the primitive ancestor of the Vertebrates had a central nervous
system which had not separated from the epiblast in which it
was developed. As Mr. Sedgwick points out, the nervous system
in the living Vertebrates is continuous with the epiblast, and in
this respect the Vertebrate is on a par with the Cœlenterate
and the Echinoderm. It follows, then, that the limiting surface
of the neural canal in Vertebrates is part of the original surface
of the body. Now, there is one fact in the organization of a
worm which requires to be taken most seriously into account
in forming an idea of its transformation into a Vertebrate.
This fact is the pericœsophageal nerve-collar. We may sup-
pose — we must suppose — that, although in the worm-like
ancestor of the Vertebrate the nerve-cords were continuous
with the epiblast, these cords diverged to enclose the mouth,
and met again in front of it just as they do in a modern
annelid. In the Vertebrate, then, we must find a rudiment of
the original mouth within the neural canal. I believe I
have hit upon this rudiment: it is the infundibulum of the
brain. The infundibulum is a deep depression in the floor of
the neural canal which comes into the closest relation with the
hypoblast. I am not referring in the faintest way to the
hypophysis or pituitary body, which seems, according to recent
researches, to be derived from the epiblast of the actual mouth.
But the infundibulum in a Vertebrate embryo is, I believe,
actually in contact with the hypoblast in front of the notochord.
Elsewhere, along the back of the embryo, the neurochord is
separated from the hypoblast by the notochord; the notochord
ceases at the infundibulum. I fully accept all that Mr.
Sedgwick says about the elongated blastopore along the Verte-
brate back, and I would point out that Mr. Sedgwick's view
makes the back of the Vertebrate homologous with the ventral
face of the worm, just as does the Dohrnian hypothesis. But
according to Mr. Sedgwick, a supraoral portion of the nervous
system in the worm has disappeared in the Vertebrate (and in
Balanoglossus); and the present mouth and anus in the Verte-
brate are not secondary new structures but the primitive ones.
This I strenuously oppose. If we look at certain Teleostean

Fishes, such as the eel or the blennies, on Mr. Sedgwick's
view we should have a blastopore extending round nine tenths
of the longest circumference of the body, which seems to me
a reductio ad absurdum of embryology. Indeed, if, as
some writers do, we consider the closing of the blastoderm
over the ventral pole of the yolk as part of the original blasto-
pore, we have the complete circle, and the blastopore extends
all round the plane of symmetry. The anterior limit of the
primitively elongated dorsal blastopore is the infundibulum
which remains to indicate the position of the original mouth.
In the preceding number of this Journal Miss Alice Johnson
speaks of a deep pit at the anterior end of the primitive groove
in the newt at which epiblast and hypoblast are fused, and she
believes this pit to correspond in position with the future
mouth. Anyone who has studied Vertebrate embryos knows
how very late the actual mouth is in appearing ; it appears
long after the visceral clefts are wide open and the heart
beating vigorously. I am convinced that the pit observed by
Miss Johnson is the commencement of the infundibulum,
although I cannot speak from actually having traced the origin
of that structure in the newt.

All former attempts to find the original mouth ended in
placing its external opening on the actual dorsal surface, instead
of on the floor of the anterior cerebral vesicle. Dohrn, before
he wrote his ' Ursprung der Wirbelthiere ' in 1875, had
imagined that the primitive œsophagus was represented by the
pituitary body and pineal gland. In the essay I have men-
tioned, he abandons this theory for one which made the fourth
ventricle in the medulla oblongata the rudiment of the
original mouth. In his address at the British Association
Meeting in 1881, Sir Richard Owen revived the first hypothesis
of Dohrn, and stated it as an original discovery. Dohrn has
recently proved that the hypophysis represents a pair of gill
clefts. If this be true, the connection of the hypophysis with
the infundibulum explains itself on my view, because a pair of
gill clefts might have opened into the mouth.

Similarly with regard to the primitive anus I cannot accept

Sedgwick's idea that the present anus is the same structure, nor do I accept the proof brought forward by Miss Johnson on the point as far as regards the newt. The blastopore is indeed the primitive anus, but it does not coincide with the actual anus; the primitive anus was closed by the same process as that which removed the hollow neurochord from the surface of the body, and is represented by the neurenteric canal, which, in spite of Miss Johnson's failure to find it in the newt, is, I believe, never altogether unrepresented in Vertebrate embryos. Miss Johnson's researches on the primitive groove in the newt are extremely valuable as proving that there is originally a fusion of the three layers along the whole line of the primitive elongated blastopore.

The notochord was discovered long ago to originate in many Vertebrates in close relation to the hypoblast, and it was immediately concluded by morphologists that the notochord was evolved from the wall of the intestine. Some have pointed to the typhlosole in the earth-worm as the homologue of the notochord; notwithstanding that the typhlosole is a pushing in and the notochord a growth outwards. The typh· losole is represented beyond a doubt by the spiral valve in Elasmobranchs and other fishes, and has never given rise to anything outside of the intestine. Now, the notochord in the course of evolution never could have arisen from the intestine, for this reason : the dorsal aorta of Vertebrates is homologous with the subintestinal vein of an Annelid, the blood in both flows the same way, and the two have the same relations to the intestine. Therefore, if the notochord had been evolved from the wall of the intestine the aorta would in the Vertebrate have been on the dorsal side of the notochord, not, as it actually is, on its ventral side. A glance at fig. 2 of the Plate illustrating this paper will show that one might as easily suppose that in the herring the notochord was developed from the neurochord as from the hypoblast. I am not going to sustain that the neurochord gave origin to the notochord, because I think it unlikely that a nervous structure would have given rise to a skeletal one. I believe the notochord to be really mesoblastic,

and that the reason why it has such close relations with both neurochord and hypoblast is that its development in the individual has been thrown so far back, takes place so early, that the fusion between the three layers due to the influence of the primitive elongated blastopore has scarcely disappeared before the central part of the mesoblast is converted into the notochord. We have got back then to the old idea of the homology of the notochord and the three giant fibres beneath the nerve-cord in the earth-worm.

The Origin of the Vertebrate Eye.—Prof. Balfour[1] has pointed out that the eyes of Vertebrates like those of Crustacea develop as part of the thickening of epiblast which gives rise to the nervous system. Prof. Lankester[2] has inferred from the relations of the cerebral eye in Ascidians, that the ancestral Vertebrate was transparent and had eyes on the floor of the brain cavity. But Sedgwick's revelation enables us to go a step further in tracing the evolution of the Vertebrate eye. In the ancestor of the Vertebrate before the neural canal had begun to form, two eyes existed somewhat in front and at the sides of the mouth actually in the region of the central nervous system. There is no impossible assumption in this; the eyes of Cœlenterates in the present day are in contact with the superficial nervous system, and probably eyes in nearly all cases existed in the same relation before the nervous system was separated from the epiblast. These eyes in the ancestral Vertebrate were open cups like those of a modern Patella, Haliotis, &c. When the nervous system formed a canal it covered over these eyes, which were then open to the neural canal, to the cavity of the anterior cerebral vesicle in the floor of which was the original mouth. The animal was probably at this time transparent, and light reached the simple eyes both through the roof of the cerebral vesicle and the sides of the head. Now, as the walls of the cerebral vesicle increased in thickness, and perhaps became more opaque than the rest of the body, the

[1] 'Report of Brit. Ass. Meeting,' 1880, " Address to Department of Anat. and Phys.," Sec. D.

[2] " Degeneration," 'Nature,' Series 1880.

eyes would be affected in greater proportion by the light
passing through the side of the head. For this reason the eye
cups would deepen and begin to grow out from the cerebral
vesicle in order (as already pointed out by Lankester) to
get nearer to the lateral surface of the head. They would
ultimately come into contact with the epiblast at this surface.
The part of the epiblast with which the optic cups came
into contact has remained transparent while the rest of the
body has become opaque. The reason why the crystalline
lens is now formed as an invagination is easily explained.
The epiblast at this region became thickened in order to
act as a lens. The lens was ultimately perfected by being
converted into the biconvex shape and removed from the body
surface. The separation of the retina from the crystalline
lens is also comprehensible, for it was an improvement of the
optical apparatus at every step.

I do not intend here to discuss the rationale of the origin
of the new mouth, of the visceral clefts, or of the actual anus.
Although I believe these to be new structures I am not satis-
fied as to their explanation. Sedgwick regards visceral clefts
as serially homologous with segmental organs, but I have not
considered the subject sufficiently at present to have a definite
opinion. I will conclude with a few words as to segmentation.
Sedgwick's interesting theory does not at all explain the
peculiarity of the growth of segmental animals, namely by the
formation of new segments between the last and the end of the
body. But this mode of growth is another difficulty in the
way of his view of the blastopore. For he supposes the planes
of segmentation to be originally perpendicular to the direction
of the blastopore, and yet if the actual Vertebrate anus is the
end of the blastopore, the planes of segmentation in the Verte-
brate tail are parallel to the direction of the blastopore.

EXPLANATION OF PLATE I.

Illustrating Mr. J. T. Cunningham's memoir on " The Signifi-
cance of Kupffer's Vesicle, with remarks on other Questions
of Vertebrate Morphology."

LETTERS OF REFERENCE.

Bl. Blastopore.   *Ep.* Epiblast.   *Gc.* Gastrula-cavity.   *Hy.* Hypoblast.
In. Infundibulum.   *Kv.* Kupffer's vesicle.   *Me.* Mesenteron.   *Mes.* Meso-
blast.   *Ne.* Neurochord.   *Ot.* Otocyst.   *No.* Notochord.   *Pe.* Periblast.
Yk. Yolk.

FIG. 1.—Embryo of herring fifty-four hours after fertilization.   Obj. A oc.
3, Zeiss.

FIG. 2.—Section of a herring's embryo at stage represented (fig. 1).   All
the sections were cut perpendicular to both ends of the embryo.   The figure
gives a section of the posterior part of the embryo taken near the extremity.

FIG. 3.—More anterior section of the same series, passing through Kupffer's
vesicle.

FIG. 4.—Section a little more anterior of the same series.

FIG. 5.—From a herring's embryo, sixty-three and a half hours after fertili-
zation, about the region where Kupffer's vesicle was at the previous stage.

FIG. 6.—Section passing through otocysts, same stage as fig. 5.

FIG. 7.—Herring embryo sixty-two and a half hours after fertilization.

FIG. 8.—Diagram of a section through the plane of symmetry of a herring
embryo at the stage of Kupffer's vesicle.

FIG. 9.—Diagram of a section through the plane of symmetry of a frog
embryo at the stage corresponding to that of the Teleostean in fig. 8.

N.B.—All the sections were drawn with the same lenses, the outlines by
means of Abbé's camera lucida.   The scale of $\frac{1}{100}$th mm. is given in the
plate.

Fig. 1.

Ne

Fig. 2.

Ne

Pe.

Yk.

No

Kv

Yk

Ep.

Fig. 4.

No.   Yk

Pe

Me.

Ot

Fig. 7.

Yk

Fig. 8.

Yk.

Hy

No

K.v

J T Cunningham del.
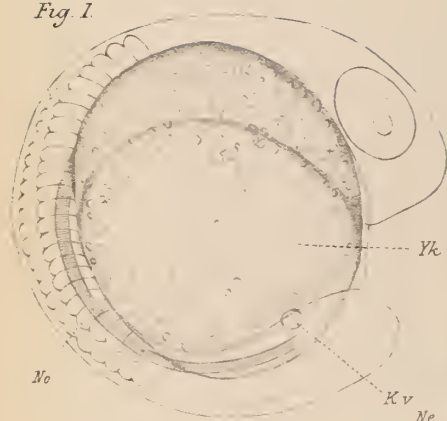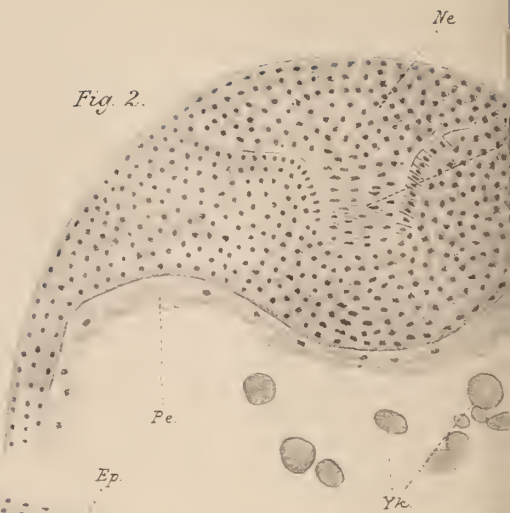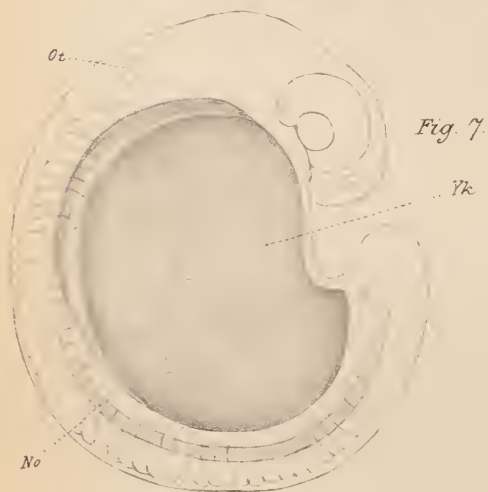
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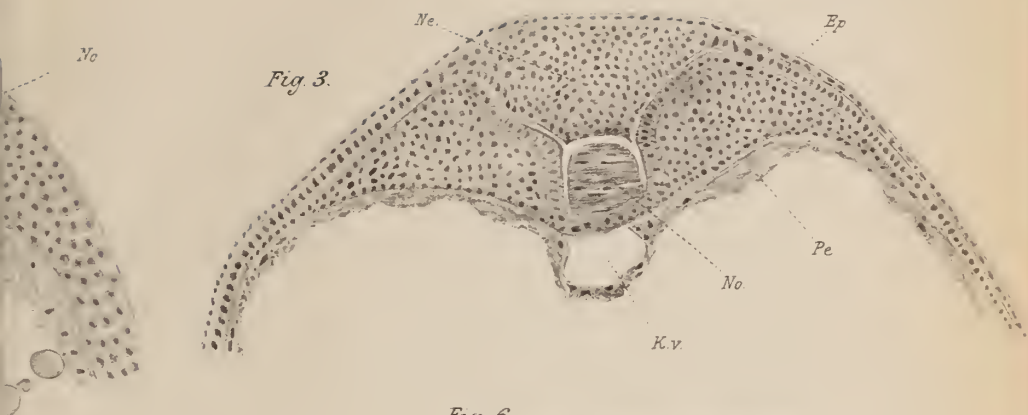
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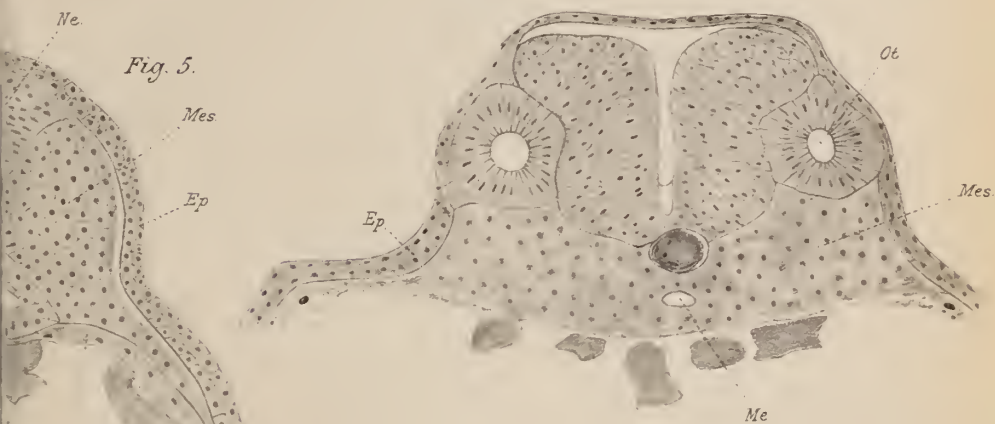
Fig. 5.

Fig. 9.
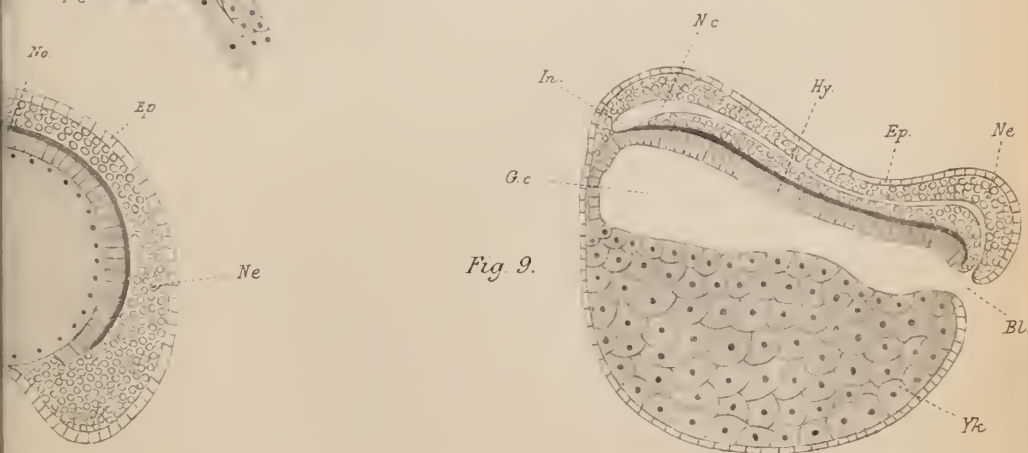
Blastopore, Mesoderm and Metameric Segmentation.

By

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Fellow of Caius College, and Balfour Student in the University of Cambridge.

With Plate II.

A YEAR ago my observations on the development of certain forms of invertebrate animals suggested an explanation of the behaviour of the blastopore, and led me to consider the various speculations concerning the middle germinal layer put forward in recent years. The theory which I thus deduced embraces the question of metameric segmentation. On my voyage to Australia I wrote the present paper. Meanwhile my friend Mr. Sedgwick, to whom I had written some of my conclusions, was preparing a contribution to the same subject. Since then Mr. Sedgwick's paper[1] has appeared, and I find not only that my conclusions as to the meaning of segmentation are fundamentally different, but also that he leaves the larger question of mesoderm untouched. The origin of mesoderm in Phoronis was the starting-point of my inquiry. I shall first describe the facts in this animal, and I would point out that my discoveries are due entirely to the facilities for investigating minute embryos afforded by my method of obtaining automatic series of sections. I failed to observe the details which are contained in the present paper in my original sections from which a preliminary account ('Proc. Roy. Soc.' 1882) was composed.

[1] 'Quart. Journ. Mic. Sci.,' 1884, " Origin of Metameric Segmentation."

DEVELOPMENT OF PHORONIS.

The details of the segmentation of the ovum are not required in this paper. A planula slightly oval in form is the final term of the process. The long axis of this planula coincides with the future long axis of the gastrula. One half of the cells are large (endoblast), the other half are small (ectoblast).

GASTRULATION.

The gastrula is formed by invagination. The first sign of this is the flattening of the endoblast-half of the oval planula. The sides begin to grow over the endoblast, and this takes place in such a way that the saucer-shaped structure is deepened towards the future anterior end (Pl. II, fig. 1). The anterior end also grows rapidly over the endoblast, thus early indicating the future præoral lobe. The bilateral symmetry is thus clearly marked. Posteriorly the sides fold over so as to meet in the middle line (Pl. II, fig. 9). The cavity of the archenteron is now sufficiently large to form lips to the blastopore. Quite posteriorly the lips completely fuse, so that during the gastrulation the extreme posterior portion of the archenteric cavity is obliterated (Pl. II, fig. 10). It is represented by a fused solid mass of cells (r). The lips of the blastopore continue to approach the middle line, and as they touch fuse with each other. This fusing proceeds from behind forwards. The blastopore has in this way become divided into two parts exactly comparable to the parts long known in some vertebrates (Pl. II, fig. 2). I shall speak therefore of the posterior portion as the primitive streak, and the groove along the line of closure as the primitive groove. The invagination has now produced a gastrula with an opening situated in the anterior portion of the blastopore into a large archenteric cavity (Pl. I, fig. 11). I shall now use the terms dorsal and ventral as defined by the non-blastoporic and the blastoporic regions respectively. The ventral surface now begins to grow very much more rapidly than the dorsal. The growth results in the posterior point of the primitive streak becoming terminal. The exact behaviour of

the cells of the middle of the primitive streak is as follows. About the middle of the primitive streak the ectoblastic elements divide very rapidly, and very soon the primitive groove disappears in this region (cf. Pl. II, figs. 2—6). Coincidently with this a space appears between ecto- and endoblast (Pl. II, fig. 18, *v*). Consider the fate of a single one of the cells of the primitive streak. This cell is destined to give rise by division to both ecto- and endoblast. The ectoblastic portion increases more rapidly than the endoblastic, and soon the latter is no longer in contact with the former, i. e. a space has arisen in the median ventral line. This space extends both anteriorly and posteriorly. The primitive groove now only remains as a pit at the posterior end of the embryo (fig. 4, *g*). The anterior opening of the blastopore remains open and becomes the mouth of the future Phoronis, the posterior pit is destined to undergo some very remarkable changes which will be described below. During these changes the original solid mass of cells—the posterior portion of the primitive streak—remains unaltered as a cord connecting the archenteron with the ectoderm.

The Separation of the Mesoblast.

Previous Observations. — Kowalewsky originally described the mesoblast as originating in Phoronis by delamination from the ectoderm (vide Plate II, fig. 13). This mistake arose from the ectoderm cells being darker at their base. Recently Metschnikoff and Foettinger have attempted to solve the problem of mesoderm formation. While they have both recognised Kowalewsky's error, they have fallen into other mistakes. Metschnikoff describes some mesoblastic cells already present in the blastula stage; he figures four of them in his fig. 30. In each cell Metschnikoff has drawn a nucleus. I have frequently observed this appearance of cells. It is caused, however, by the amœboid processes of the endoderm cells growing into the segmentation cavity. This is easily proved by making real sections. Another possible explanation of Metschnikoff's account may lie in the presence of certain peculiar bodies in

the early gastrula stages.   In the endoderm cells little spherical
masses of apparently the same material as the body of the cells
themselves are frequently found.   Each little mass in hardened
embryos is separated off by a clear space.   I have traced these
bodies from their birthplace into the body cavity.   They
never possess a nucleus, and they disappear at a very early age.
Their significance remains unknown to me unless they be
merely an excess supply of nutriment analogous to food yolk.
Foettinger has arrived at somewhat extraordinary results.   He
says, "J'ai non seulement constaté l'existence des premiers
elements-mesodermiques à des stades plus jeunes que celui
signalé par Metschnikoff, mais encore je crois pouvoir reculer
leur première apparition jusqu'à l'œuf en voie de segmenta-
tion."   The bodies referred to the mesoblast are, I believe,
either due to the reagents used in preparing the embryos, or
are the bodies referred to above.   I have observed them fre-
quently, but it is certain that they have nothing to do with
the true mesoblast, whose origin I shall now describe.

Before the lips of the blastopore meet there is no meso-
blast (fig. 1).   When the closing of the blastopore has already
extended sufficiently far forwards to shut off a small archen-
teric cavity, two pouchings of the endoderm occur on either
side of the blastopore (fig. 8, ad).   Each pouch is longi-
tudinally extended in the direction of the long axis of the
body, and is deeper towards its anterior end.   The endoderm
cells covering the region of the pouch now undergo some
division (fig. 8), and a mass of cells is budded off on either
side (me').   These cells as they are formed arrange them-
selves into a sac enclosing a cavity (fig. 17, ad).   These cavities,
however, never communicate with the cavity of the gut.
The pouch of the endoderm is soon obliterated, and the
cells return to the size of the other endoderm cells.   The
hind part of each pouch lies about opposite to the most anterior
point where the lips of the blastopore have closed.   On
either side of the primitive streak a few mesoblast cells are
budded off from the cells forming the primitive streak (fig.
18, me'').   Behind the primitive groove becomes deeper, and

this deepening continues after the middle part is obliterated (figs. 4, 6, 11, and 12). The deepening of the groove soon forms a very definite pit (*g*). This pit, when by the growth of the ventral surface it has become nearly terminal, grows into two pouches which project into the cavity between the skin and gut on either side of the solid cord of cells, which is the persistent hind part of the original primitive sheath (fig. 13 and fig. 14, *pd*). These pouches are derived from cells homologous with those which have already given rise to mesoblast. The continuity of this posterior pair of pits with the anterior is kept up by the few cells (*me″*) budded off in the middle of the primitive streak. The same growth which opened up the space between ectoblast and endoblast has separated the anterior and posterior mesoderm. The fact that in Phoronis the two ends of each mesodermic pouch are actually connected by an intermediate cord of cells depends on this formation of a primitive streak along the whole line of closure of the blastopore.

Nephridia.

The posterior pair of mesodermic diverticula open in the middle line to the exterior. The closure of this opening proceeds in such a way that each pouch remains open to the exterior by a small pore on either side of the middle line. I believe—though this fact is not established so certainly as those above concerning the mesoderm—that each pore persists as the opening of the nephridium of its own side. The nephridia appear coincidently with the final narrowing of the mesodermic pores, but I have yet no sections showing the cells in the neighbourhood of the pores taking on the form of the intra-cellularly perforate excretory cells. The formation of these excretory cells, which lie in a blood space of the splanchno-pleure and not in the body cavity, I have independently traced from the mesodermic cells of the posterior pouches.

Anus.

Meanwhile the remnant of the primitive streak, the posterior solid cord of cells, opens up, and forms a canal leading from the archenteron to the exterior (fig. 15). The alimentary canal is now complete, mouth and anus having been derived from the blastopore (fig. 7, *m* and *a*).

Hypothesis.

With the help of the various morphological laws implied by such terms as precocious segregation, superlarvation, abbreviation, &c., it is possible to solve almost any morphological problem in several ways—all equally probable. The speculations which follow, I am induced to add to those already existing, not from any belief in their absolute value, but because they go in the direction of simplification. The theory which I am about to state reduces the various origins of the mouth and anus to one type. The same hypothesis gives an explanation of the various modes of origin of the mesoderm, and leads to a view of the meaning of metameric segmentation which, so far as I know, has not been hitherto suggested.

Given a gastræa already become bilaterally symmetrical by the elongation of the blastopore and the differentiation of anterior inhalent and posterior exhalent currents, and in which the main development of organs takes place around the mouth, so that the mesoderm thus resulting comes to lie in development as two masses of cells on either side of the body.[1]

I propose to show that the elongation of a long axis of the body is a possible cause of—

I. The obliteration of the relation of blastopore to mouth and anus.

II. The masking of the original mode of mesodermic formation.

III. Metameric segmentation.

[1] Whether the mesoderm originally arose as diverticula or not does not concern the present speculation.

I. The Obliteration of the Relation of Blastopore to Mouth and Anus.

Previous Observations.—Since the time of the Gastræa Theory many writers have occupied themselves with the blastopore. Lankester (This Journal, 1877), in accordance with his planular theory, came to the conclusion that the coincidence of mouth and anus with the blastopore was only a developmental convenience. He says (loc. cit.), "Regarding, as I do, the blastopore as an orifice of a secondary nature, existing solely in relation to the invagination process, and originating after mouth and anus had made their appearance in the progress of animal evolution, I seek to explain its occasional relation to the mouth and to the anus as cases of adaptation." Balfour, after enumerating the different fates of the blastopore in the animal kingdom, says, "It is clearly out of the question to explain all these differences as having connection with the characters of ancestral forms. Many of them can only be accounted for as secondary adaptations for the convenience of development." The number of groups in which a slit-like blastopore has been described is very considerable (vide Balfour, vol ii, p. 282). Lankester was the first to suggest that a slit-like blastopore which might close at either end would, if taken as the ancestral type, account for the various fates of the blastopore in molluscs. Hatschek, in his paper on Teredo, has suggested the possibility of phylogenetically deriving the anus which arises in this animal; secondarily, as an ectodermic invagination from part of a slit-like blastopore. But he bases this view on the fact that the anus corresponds in position with the hind wall of the gastrula mouth. Metschnikoff, in combating Hatschek's views on the early expression of bilateral symmetry, has denied the ancestral character of the slit-like blastopore. He says, " Kann man dem geschlitzten grossen Blastopor keine palingenetische Bedeutung zuschreiben und muss ihn als eine embryonale Anpassungs-erscheinung ansehen." Sedgwick (loc. cit., p. 27) concludes that " the mouth and anus of the Triploblastica are

derived from the primitive mouth." I fail to see how his theory on p. 34 explains the behaviour of the blastopore. In the first place his conception of the blastopore is different from that used in the present paper. Page 35 he writes, "Consequently the only course open is that the mouth should be formed as a secondary perforation entirely independent of the blastopore." Sedgwick's theory is contained in the following passages (p. 34) : "My view is that in those animals in which it does not give rise to the mouth and anus, it functioned as the larval mouth while the animal was developing, and persisted until parts of the embryo were developed between it and the position of the mouth and anus of the adult, which parts had arisen in the phylogenetic history in the adult after the primitive mouth had completely divided into the mouth and anus. These parts never had been traversed by the original slit-like mouth, because they had appeared at a stage in evolution subsequent to the stage in which the mouth and anus were one. It cannot therefore be a matter of surprise if the blastopore does not elongate and bisect these latter structures, which never had in the history of the animal been perforated by the blastopore." I ask, how have the cells which are to form mouth and anus anything to do with blastopore ? My hypothesis is as follows.[1]

The behaviour of the blastopore in Phoronis is obviously due to the attainment of a terminal anus. Suppose the long axis of the body to increase still more rapidly while the posterior part of the blastopore still remains terminal. Suppose in the early stages of development the importance of a complete alimentary canal is not equal to the importance of the body form, then the tendency of the endoblast to divide into anterior and posterior portions attached to anterior and posterior parts of the blastopore respectively might be consummated. The behaviour of the cells in the middle of the primitive streak of Phoronis, which resulted in the opening of a space between endoderm and ectoderm, would tend to begin at an earlier stage.

[1] Delamination need not be discussed, since the existing hypotheses (vide Balfour, 'Embryology,' vol. ii) are sufficient to bring the case under my theory.

What would be the structure of a planula when this influence has reached back to the preinvaginated stages?

The ectoderm cells would grow in from either side, and encroach on the solid mass of endoblast until this (i. e. endoblast) would be completely divided into anterior and posterior masses. The endoblast may be divided in this way into any number of separate portions (cf. below metameric segmentations). Let invagination now take place; each mass of endoblast will be invaginated. The invaginated endoblast will grow according as it has been divided into endoderm or mesoderm; such division may have taken place in a great many ways (vide mesoderm below); when the anterior endoblastic mass is the larger we have a so-called oral blastopore, e. g. Pilidium, Phascolosoma, and Phoronis, according to previous observers; when the posterior is the larger we have an anal blastopore, e. g. Paludina, Serpula, and Echinodermata. Let the invagination of the different endoblastic masses cease to be synchronous, and the primitive relations will become still more marked. The extreme cases are described as stomodœa and proctodœa. The difference between Sedgwick's view and my own consists in the fact that I suppose that portions of the blastopore actually exist beyond the " parts which, in the phylogenetic history in the adult, had arisen after the primitive mouth had completely divided into the mouth and anus."

II. The Masking of the Original Mode of the Origin of Mesodermic Formation.

In Phoronis the original pair of diverticula are almost divided into two pairs. The anterior pair produce only a small proportion of the future mesodermic structures. Argiope is an instance of the anterior mesoderm being large. No posterior mesoblast has yet been described in this form. The mesenchyme of the præoral lobe of the Hertwigs is the same anterior mesodermic diverticula reduced in the opposite direction. In Phoronis the anterior mesoderm would be described as of endoblastic origin, the middle as originating at the lips of the blastopore, while the posterior pouches would be assigned to the ectoblast. But the connec-

tion between these three methods, though obvious in Phoronis, has not been explained in the same way in the rest of the Triploblastica. The six diagrams (fig. 19) represent six different modes of formation; they may all coexist in the same animal. Thus, in Phoronis we find those represented in the diagram by 3, 4, and 6, and in the Chick and other Vertebrates 4 and 6.

III. Metameric Segmentation.

In Phoronis the elongation of the blastopore produces two pairs of masses of mesoblast, each of which might be regarded as constituting a "mesodermic somite;" but in Phoronis the first long axis developed is not to be the long axis of the adult. The long axis of an adult Phoronis is exactly at right angles to that of the larva. The further slight extension of the larval long axis is thus able to proceed pari passu with the growth of the posterior pair of mesodermic pouches. In Chætopoda, Arthropoda, and Vertebrata the long axes of adult and larva are identical. The elongation of the body in these forms takes place before the mesoderm grows. The same cause which separated the mesoderm in Phoronis operates during a much longer developmental period. The mesoderm cannot keep pace with the ectoderm. It must therefore be left to afterwards complete its growth. The various positions in which it may remain give rise to the various origins of the mesoderm. Take Amphioxus, where the mesoderm has remained entirely in the endoblast. Here we have a regular elongation of the body taking place when the mesodermic cells are still undifferentiated. The mesodermic diverticula are regularly drawn out, and as regularly they leave small portions of the whole in front. Hatschek has described a shallow groove connecting the separated diverticula on each side, which is explained by the present hypothesis. I take it to be of the same nature as the connecting strand of mesoderm in Phoronis. Echiurus (Hatschek) is a form where the greater part of the mesoderm remains near the posterior pole of the long axis as in Phoronis. As the long axis grows the mesoblast has to be left

in formative masses, which afterwards grow to line the body cavity (in Echiurus, however, the mesoderm much more nearly keeps pace with the ectoderm than in Amphioxus). Thus, I consider that in Chætopoda, Arthropoda, and Vertebrata the mesoderm will tend to be left in regular pairs of masses, while the elongation of the body is taking place; I seek to explain the whole of the facts of metameric segmentation as arising from the necessities of development.

In Phoronis the external openings of the nephridia are parts of the blastopore. The same considerations which have been applied to the mouth and anus, mesoderm and metameric segmentation bear on the question of the origin of nephridia. The nephridial portions of the mesoderm may remain in various positions, in other words, the nephridia may in accordance with my hypothesis arise as single or as serial ectoblastic or endoblastic pairs of pouches with or without connecting longitudinal canals or cords.

Instances of most of these possible modes of origin have been described in the different groups of animals. The bearing of the above facts and hypotheses on the nervous system, gill-slits, notochord, and other organs, will be obvious to anyone who has followed me so far. I hope to be excused from entering into completer discussion of my hypothesis by reason of the want of books of reference in my present situation out of the world.

Summary.

Facts in the development of Phoronis—

1. The blastopore gives rise to both mouth and anus.

2. The mesoderm arises in an anterior pair of endoblastic modified diverticula, and in a posterior pair of ectoblastic diverticula connected by a few mesodermic cells derived from the middle of a primitive streak.

3. The nephridial openings to the exterior are parts of the blastopore.

Preliminary interpretation suggested by these facts of the development of Phoronis—

1. A gastræa with slit-like mouth and a pair of lateral
diverticula giving rise to mesoderm was the ancestor of
Phoronis.

2. The rapid growth of ectoderm in the median ventral line
nearly succeeded in destroying the continuity of the primitive
streak.

3. The necessity of an early attainment of a terminal position
by the anus caused the ectoderm to grow more rapidly than
the endoblast, and resulted in a division of the mesoderm into
anterior and posterior parts.

4. The nephridia, which might have remained either wholly
or in part with the anterior, have attached themselves entirely
to the posterior mesoderm.

Extension of this interpretation to the other Triploblastica—

1. Phoronis is the first step towards a complete division of
the blastopore. The inducing cause of such division is the
elongation of the body, while the endoblast is still in an
embryonic condition.

2. The division of blastopore caused the division of meso-
derm.

3. The division of mesoderm results in—

i. The masking of the original mode of mesoderm forma-
tion.

ii. Metameric segmentation.

In Camp, Burnett River,
        Queensland; July 27, 1884.

————————

EXPLANATION OF PLATE II,

Illustrating Mr. W. H. Caldwell's paper on "Blastopore, Mesoderm and Metameric Segmentation."

List of Reference Letters.

Blastopore, *bl.* Mouth, *m.* Anus, *a.* Primitive streak, *p. s.* Primitive groove, *p. g.* Posterior pit, *g.* Posterior solid cord, *r.* Anterior mesodermic diverticulum, *a. d.* Posterior mesodermic diverticulum, *p. d.* Mesoderm, *me.* Præoral lobe, *p. l.* Archenteron, *ar.* External opening of nephridium, *n.* Body cavity (cœlom), *c.* Splanchnopleuric vascular space, *v.* Nutritive (?) body, *x.*

All the figures, both of whole embryos and of sections, were drawn by means of Zeiss's two-prism camera, from permanent preparations in Canada balsam. The embryos were treated as follows:—Mixture of two volumes corrosive sublimate + one vol. glacial acetic acid for 1 second, water 15 seconds, alcohol, 50 per cent., 5 minutes, alcohol 70 per cent.

Figs. 1—7. Zeiss's oc. 2, obj. D. Figs. 8—18. Zeiss's oc. 2, obj. 1-12th homog. imm. All the embryos belong to the species of Phoronis, living in the harbour of Naples in dense colonies without sand adhering to their tubes, except that of which Fig. 13 is a section. This is an Australian Phoronis, discovered in Port Jackson by Mr. Haswell, of Sydney University. The sections drawn are taken from complete series. Each section is ·005 mm. thick. I have series ·0025 mm. thick. These, though necessary for observation purposes, are not so convenient for drawing.

Figs. 1—4.—Four embryos, showing mode of closure of the blastopore.

Fig. 1. Blastopore, *bl.*, before meeting of lips.

Fig. 2. Blastopore fused posteriorly: open part=mouth, *m.*; closed part= primitive streak and groove, *p. g.*

Fig. 3. Primitive groove, *p. g.*; disappearing præoral lobe, *p. l.*

Fig. 4. Primitive groove enlarging into pit, *g.*, posteriorly; præoral lobe, *p. l.*

Figs. 5 and 6.—Two embryos viewed from the left side. Præoral lobe, *p. l.*; primitive groove, *p. g.*; mouth, *m.*; posterior pit, *g.*

Fig. 7.—Older embryo with complete alimentary canal. Mouth, *m.*; anus, *a.*; external opening of nephridium, *n.*

Figs. 8—10.—Three sections in a nearly transverse plane of an embryo in a stage between that of Fig. 1 and that of Fig. 2.

Fig. 8. In front of mouth. Anterior mesodermic diverticula, *a. d.*; anterior mesodermic cell, *me.*; archenteron, *ar.*

Fig. 9. Through the fusing lips of the blastopore. Primitive streak, *p. s.*; archenteron, *ar.*

Fig. 10. Through the posterior part of the primitive streak. Primitive groove, *p. g.*; posterior solid cord of cells, *r.*

FIG. 11.—Median section, longitudinal vertical, through a slightly older embryo than Figs. 8, 9, and 10. Præoral lobe, *p. l.*; anterior mesoderm, *me'.*; mouth, *m.*; archenteron, *ar.*; posterior pit, *g*; posterior solid cord of cells, *r*; Nutritive (?) body, *x*; body cavity, *c.*

FIG. 12.—Embryo slightly older than Fig. 4. Median longitudinal vertical section. Præoral lobe, *p. l.*; anterior mesoderm, *m'.*; body cavity, *c.*; vascular space, *v.*; middle mesoderm, *m''.*; posterior mesoderm, *m'''.*; archenteron, *ar.*; posterior pit, *g*; Mouth, *m.*

FIG. 13.—Embryo, Australian species, nearly same stage as Fig. 12. Longitudinal horizontal section. Archenteron, *ar.*; anterior mesoderm, *me'*; posterior mesodermic diverticula, *p. d.*; posterior pit, *g.*

FIG. 14.—Nearly same section as Fig. 13. Vascular space, *v.*; posterior pit, *g*; archenteron, *ar.*; anterior mesoderm, *me''.*; posterior diverticula, *p. d.*; posterior mesoderm, *me'''.*

FIG. 15.—Embryo, same stage as Fig. 12. Longitudinal horizontal section. Reopening of posterior cord of cells to form rectum and anus, *a.*; anterior mesoderm, *me'.*; archenteron, *ar.*

FIG. 16.—Embryo, same stage as Fig. 12. Transverse section through posterior diverticula, *p. d.*; posterior mesoderm *me'''.*; archenteron, *ar.*

FIGS. 17 and 18.—Embryos about the stage of Fig. 3. Two transverse sections. Anterior diverticula, *a. d.*; body cavity, *c.*; vascular space, *v.*; primitive streak, *p. s.*; primitive groove, *p. g.*; middle mesoderm, *me''.*; archenteron, *ar.*; nutritive (?) body, *x.*

FIG. 19.—Six diagrams, illustrating the typical modes of mesoderm formation, *e. g.*

1. Peripatus, cf. Hertwig's " Cœlom-theorie," Pl. ii, fig. of insect embryo.
2. Amphioxus.
3. Nemertine larva of Desor (?), Phoronis (posterior).
4. Pristiurus, Phoronis (anterior).
5. Lopadorhynchus (Kleinenberg).
6. Primitive streak region, many Vertebrates and Phoronis.
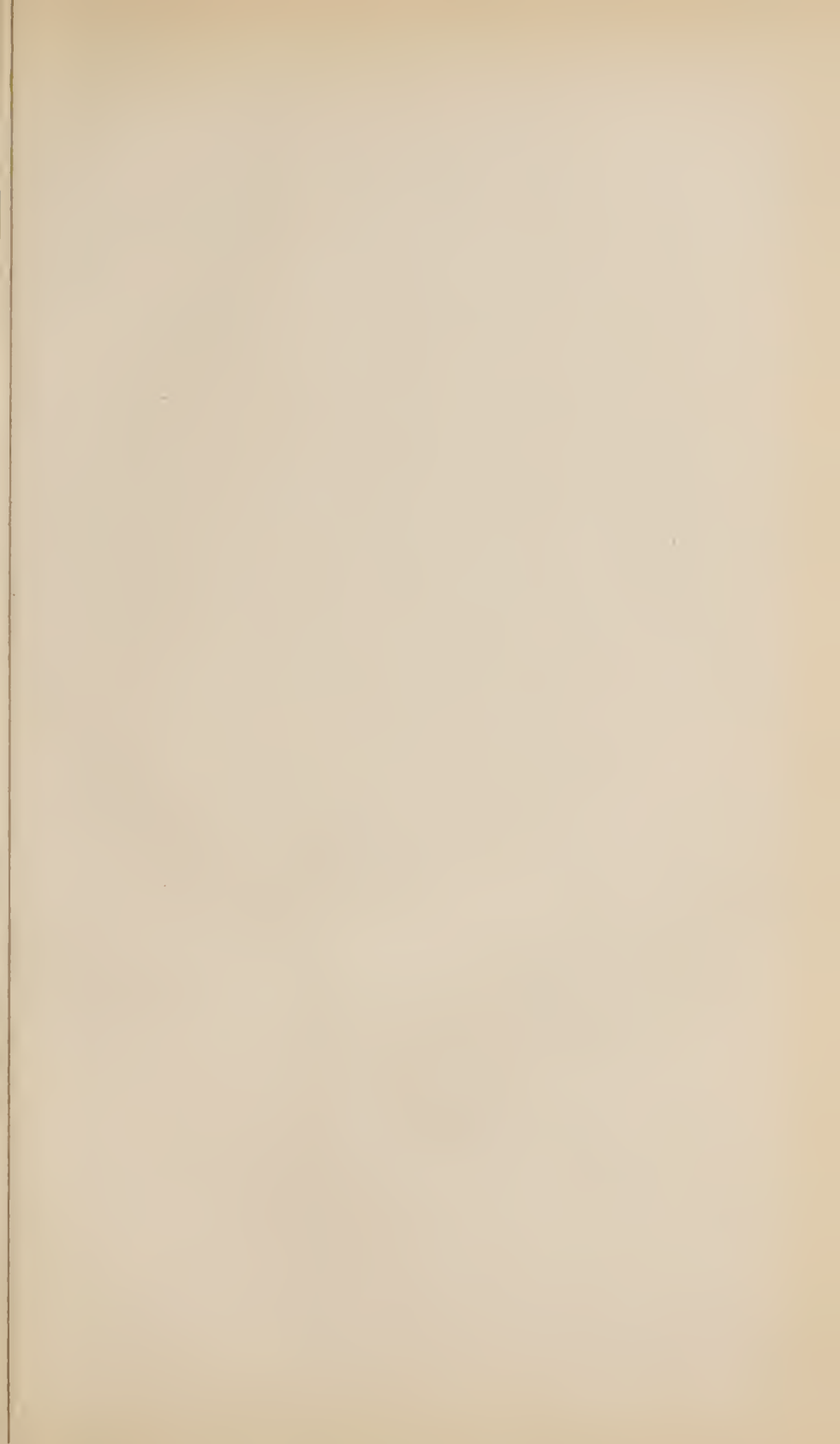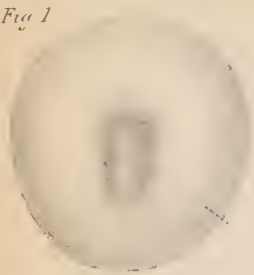
Fig. 1   Fig. 2   Fig. 3
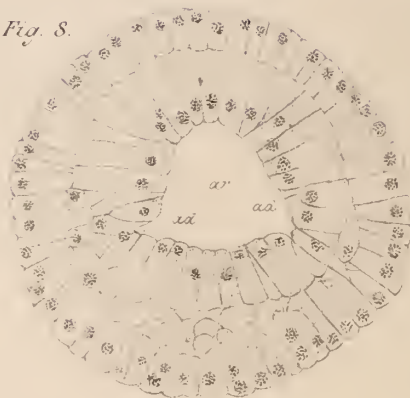
Fig. 7   Fig. 8   Fig. 9

Fig. 12   Fig. 13

Fig. 16   Fig. 17   Fig. 18
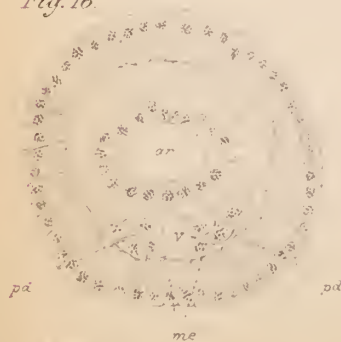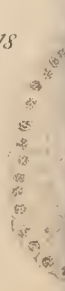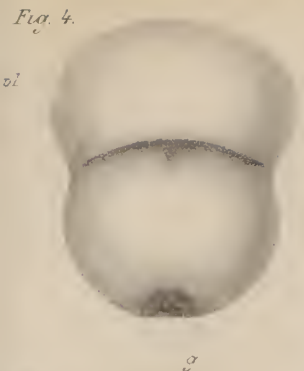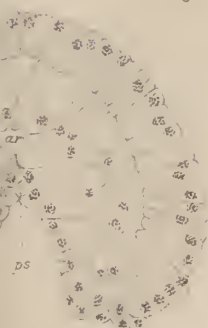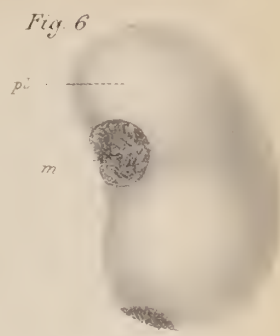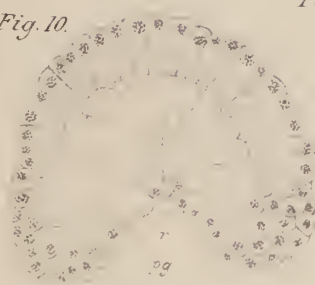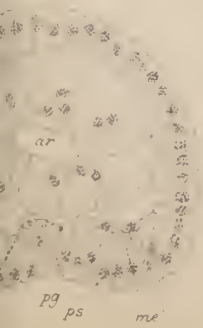
On the Origin of the Hypoblast in Pelagic Teleostean Ova.

By

George Brook, F.L.S.

With Plate III.

In a paper which I brought before the recent meeting of the American Association for the Advancement of Science, held in Philadelphia, attention was called to certain points of difference in the development of pelagic and non-pelagic teleostean ova. After the meeting I had an opportunity of seeing a paper then just published by Agassiz and Whitman (1), and when in Cambridge Dr. Whitman very kindly showed me his beautiful series of drawings on this subject. The conclusions arrived at by Dr. Whitman are so completely at variance with my own on some points, that it appears necessary for me to give a detailed account of my observations with carefully made drawings from actual sections of the egg. I may here add that whereas in very small pelagic ova I have found an optical section of the living egg to give fairly reliable details of the process of segmentation, invagination, &c., I have found that in larger eggs this method is not to be relied on at all, and that a clear interpretation of the process of invagination can only be obtained from actual sections made from eggs taken at frequent intervals.

The form which I have studied most, and from which most of my sections were prepared, is Trachinus vipera, a general account of the development of which will be found in 'Linn.

Soc. Journ. Zool.,' vol. xviii, pp. 273—290, and unless other-wise specified, my remarks must be considered to refer to this species.

It will be well to divide the question under consideration into two branches.

1st. The origin of the periblast (Agassiz and Whitman)= parablast (Klein); Dotterhaut (Œllacher); Rindenschicht (His); membrane intermédiaire (van Bambeke); yelk-hypoblast (Ryder); and intermediary layer of American authors.

2nd. The part played by the periblast in the process of invagination.

It has usually been considered by authors that whatever might be the ultimate relationship between the blastodisc and the periblast, one thing was clear at least, that they originated independently of each other. Hoffmann (2) asserts that he has actually seen the first cleavage process take place, and that the first "spindle" divides equatorially, dividing the egg into two parts, the germinal disc and the food yolk with a thin layer of protoplasm separating the yolk from the germinal disc, and that this thin protoplasmic layer becomes the peri-blast. Agassiz and Whitman, however, assert that the first cleavage process is meridional, and that the periblast is after-wards formed from the marginal cells of the segmenting disc, which, when once separated, never again unite with it. I cannot say from actual observation in which direction the first cleavage is made, but in Trachinus the periblast arises inde-pendently of the germinal disc. As the thin protoplasmic layer settles down to the lower pole of the egg, the majority of it is included in the first two cells of the blastodisc. As if not to waste any material the remainder collects around this disc, and is afterwards developed into the periblast. I have sometimes observed as early as the two-cell stage, when seen in optical section, a thin granular layer of protoplasm under the blastodisc; and in later stages I have sections showing a lower lens-shaped mass of cells (the lentille of van Beneden) differing altogether in structure from those above, and which possibly forms a central portion of the periblast, but this is

not clear, and the lower margin of the blastodisc becomes quite flat again before the marginal periblast is pushed under it. The gradual development of the periblast (there called the intermediary layer) in Trachinus has been shown in my paper already referred to, and it is not necessary to repeat it again here. About the time free cells are formed in it, a section has the appearance shown in fig. 1. The epidermal layer of the epiblast is already differentiated, and there is no segmentation cavity; nor, so far as I can make out, does the periblast extend quite under the disc. As early as the sixteen-cell stage I have noticed that the central cells of the disc do not lie on the yolk, so that there is a shallow cavity between the disc and the yolk in its central area; but this becomes filled up as segmentation goes on, and does not represent the true segmentation cavity in which the hypoblast is formed. As to whether the nuclei in the periblast arise by true free cell-formation or are derived from a subdivision of the lower part of the first cleavage spindle I cannot say, but in Trachinus, at least, there appears to be no doubt that they do not come from the margin of the germinal disc, as Whitman asserts is the case in Ctenolabrus. If, as Hoffmann asserts, the first cleavage spindle formed of the male and female pronuclei really does divide at right angles to the axis of the egg so as to form at the outset two layers, his archiblast and parablast (non Klein), it would appear more probable that the nuclei found later should be the result of the subdivision of the original nucleus of the layer than that they should arise independently. In Trachinus and Motella the ring of periblast gradually becomes more granular before cells appear, the granules cluster together in groups, and it has certainly appeared to me sometimes as if free cell-formation really did take place. Nothing short of a careful investigation of this stage in a large number of different forms may be expected to settle this question definitely.

Next as to the origin of the hypoblast, Hoffmann and Messrs. Agassiz and Whitman, though differing in their ideas as to the origin of the periblast, are all equally confident that this layer

takes no part in the formation of the hypoblast. Hoffmann
asserts that the invaginate layer of the ring and embryonic
shield is split off from the archiblast as a p r i m a r y e n t o -
d e r m, and that this afterwards is differentiated into the meso-
derm (several cells deep) and the secondary entoderm
(only one cell deep). Henneguy[‡] also states that in the
trout the invagination is caused by an infolding of the
segmented disc upon itself, and that, as the invagination pro-
gresses, the lower invaginated portion remains quite separate
from the original upper portion, and that indeed he can demon-
strate a space between them. On the other hand, van
Bambeke (11), Klein (8), Kupffer (7), and van Beneden (3),
agree that the hypoblast is derived from the periblast, while
Balfour (10), Ryder (6), Kingsley and Conn (9), and others,
admit that the periblast plays a more or less important part in
the structure of hypoblastic tissues. Haeckel (5) failed to
recognise the periblast altogether, and his diagrams of the
invagination of the hypoblast can scarcely be true for any
Teleostean. The eggs studied by Haeckel were pelagic and
supposed to belong to a species of M o t e l l a ; but I think there
must be some mistake here, as I have studied the development of
M o t e l l a m u s t e l a and can confidently assert that the whole
process of development is different from that given by Haeckel.
With a view to studying the process of invagination in
T r a c h i n u s, I have preserved eggs at half-hourly intervals from
a little before the appearance of the blastodermic rim until
this was quite well-defined all round the disc, a period of deve-
lopment occupying four hours at a temperature of about
62° Fahrenheit. The eggs were prepared by the picrosul-
phuric acid method and stained with cochineal. It is on
sections made from these eggs that my conclusions are based.
Figure 2 represents a section of one of the earlier of these
stages, in which the periblast is seen to ·be collected around
the margin of the disc and to have pushed itself some little
way underneath ; but there does not yet appear to be a layer
of periblast on the floor of the segmentation cavity. The
epidermic layer of the epiblast is, however, well differen-

tiated and has grown down over the periblast, as is shown in the figure. An hour and a half afterwards (fig. 3) the periblast is seen to have pushed its way completely across the floor of the segmentation cavity, and now contains quite a number of free nuclei and cells. The blastodisc in spreading over the yolk has thinned out somewhat, but there is still no sign of invagination. Half-an-hour later (fig. 4), the cells in the periblast have accumulated under the rim of the blastodisc; on the left hand of the figure these cells are seen to be quite round and arranged in a row ready to take their places alongside the lowest layer of the blastodisc. The right hand of the same figure shows an abnormal form of the periblast in which the cells, nuclei, and surrounding protoplasm have been withdrawn into a pocket in the yolk, possibly caused by shrinking in the hardening process. The epidermic layer is still seen to reach some way over this mass of periblast. Half-an-hour later still (fig. 5), the periblast again covers the floor of the segmentation cavity more thickly, and free nuclei are to be observed rising from the yolk to help in building up this layer. The first row of hypoblastic cells are now seen attached to the lowest layer of the disc, but are recognised by their round clear outline. A little later again (fig. 6) two rows of hypoblast cells are seen in their proper place, and the living egg in this stage shows a clearly defined ring with the beginning of the prominence to form the embryonic shield. The outline of the original blastodisc is well marked off, and the new cells are sufficiently different in shape to mark off where one layer begins and the other ends. The epidermic layer is still seen in its original position and has taken no part in the process. The new layer is now formed rapidly, and nuclei and free cells are seen crowding up from the yolk to help in this work. Fig. 7 represents a little later stage in the embryo of Motella mustela seen in optical section where much the same process has been at work. Here, however, the resulting cells of the new layer are so much larger and clearer in outline than is usual in Teleosteans that the line of demarcation between the old and new layers is quite distinct from

one end to the other. Free nuclei are also seen both in the yolk and on the floor of the segmentation cavity. Although earlier phases of the hypoblast were not observed in Motella, it seems impossible that such large and well-defined cells could be the result of invagination from the cells of the archiblast, when the cells of the latter are scarcely distinguishable under a magnifying power of 100 diameters. When, however, we have the data arrived at from a study of Trachinus to work upon, there is no difficulty in accounting for their origin.

If, now, my figures, which are carefully copied from actual sections (excepting figure 7), be compared with those of van Bambeke, Klein, and van Beneden, it will be seen that our observations agree very closely. Indeed, van Beneden's fig. 9 (loc. cit.), which also represents a pelagic egg, shows the identical process at work which I have described for Trachinus. The question then arises, is the hypoblast formed by a true process of invagination? It is quite true that the rim grows from the margin inwards because the cells from the periblast commence at the margin close to the overlapping epidermic layer and are filled up from within. Is not something more than this meant by invagination? I take it that invagination in the true sense means an ingrowth or an infolding of a layer already existing, the archiblast. If this be so, there is no true invagination in such pelagic ova as those here described, and the hypoblast is not derived from the archiblast at all, but from the periblast and the yolk by a process of segregation.

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3. E. van Beneden.—"A Contribution to the History of the Embryonic Development of Teleosteans," 'Quarterly Journ. of Microsc. Sci.,' vol. xviii, N.S., 1878.

4. L. F. Henneguy, " Premiers phénomènes du développement des poissons osseux," ' Bull. Soc. Phil. de Paris,' 1880 ; also second notice, ' Comptes Rendus,' xcv, pp. 1297—1299, 1882.

5. E. Haeckel.—" Die Gastrula und die Eifurchung der Thiere," ' Jena Zeitschr.,' vol. ix, 1875.

6. J. A. Ryder.—" A Contribution to the Embryography of Osseous Fishes (Development of the Cod)," ' Report of Amer. Commissioner of Fish and Fisheries for 1882,' published Washington, 1884.

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9. Kingsley and Conn.—" Some Observations on the Embryology of Teleosts," ' Mem. Boston Soc. Nat. Hist.,' vol. iii, 1883.

10. F. M. Balfour.—' Comparative Embryology,' vol. ii, London, 1881.

11. Van Bambeke.—" Recherches sur l'embryologie des poissons osseux," ' Acad. Roy. de Belgique,' vol. xl, 1876.

EXPLANATION OF PLATE III,

Illustrating Mr. Brook's paper " On the Origin of the Hypoblast in Pelagic Teleostean Ova."

y. Yolk. *bl*. Blastodisc (archiblast). *ep*. Epidermic layer of the epiblast. *p*. Periblast. *s. c*. Segmentation cavity. *v*. Vacuoles in the yolk. *r*. Thickened rim of the blastoderm. r_1. Part of thickened rim included in the embryonic shield. *c*. Nuclei and cells in the yolk. c_1. Nuclei and cells loose on floor of segmentation cavity. c_2. Nuclei and cells in periblast (margin). c_3. Nuclei and cells in periblast (centre).

FIG. 1.—Transverse section of the blastodisc of Trachinus vipera taken at 7 a.m., and about twenty hours earlier than Fig. 2. × 70.

FIG. 2.—Section of Trachinus egg, shortly before commencement of hypoblast. × 120.

FIG. 3.—Section of egg, one and a half hours later than Fig. 2. × 130.

FIG. 4.—Section of egg, one and a half hours later than Fig. 3. × 130.

FIG. 5.—Section of egg, half an hour later than Fig. 4. × 130.

FIG. 6.—Section of egg, half an hour later than Fig. 5. × 130.

FIG. 7.—Optical section of a living egg of Motella mustela, showing segregation of hypoblast well advanced. × 100.

Fig. 1.

Fig. 2.
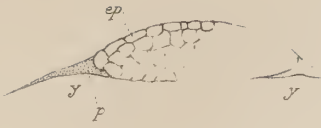
Fig. 3.

Fig. 4.
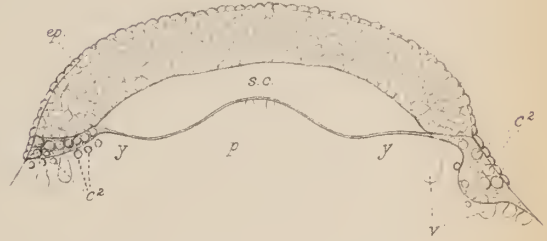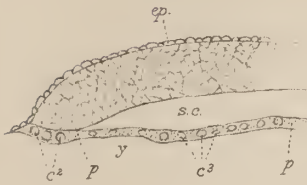
Fig. 5.
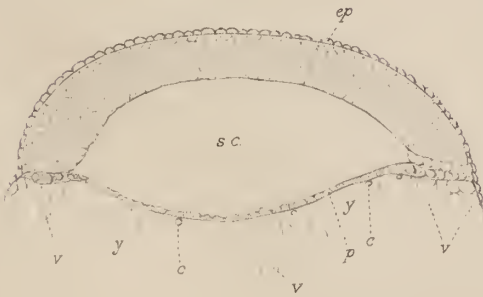
Fig. 6.
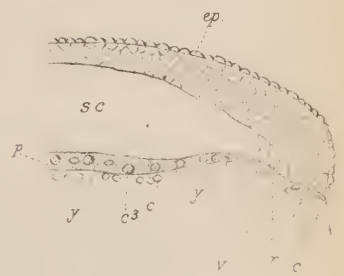
Fig. 7.

On the Presence of Eyes in the Shells of Certain Chitonidæ, and on the Structure of these Organs.

By

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With Plates IV, V, and VI.

INTRODUCTION.

ON examining a specimen of Schizochiton incisus preserved in spirit amongst a number of other animals dredged by Captain W. Chimmo, R.N., in the Sulu Sea, in H.M.S. "Nassau" in 1871, and presented by him to the Anatomical Department of the Oxford University Museum, I was astonished to remark on the shells certain highly refracting rounded bodies, arranged in rows symmetrically; they struck me at once as resembling eyes, and further examination proved that such is, in fact, their nature. On searching for eyes on the shells of other Chitonidæ I found them present in many other genera, differing, however, in each genus more or less in structure and arrangement. I published a preliminary summary of what I had been able to determine concerning these eyes in the 'Annals and Magazine of Natural History' for August, 1884. In the present paper I enter further into details, and have the advantage of elucidating my results by means of figures.

Literature of the Subject.

It is remarkable that the eyes of the Chitonidæ should have hitherto escaped notice. The main reason why they have done so is probably the fact that they do not occur, as far as I have been able to ascertain, on any common European representatives of the group such as have been ordinarily chosen for research by morphologists. Further, they are as a rule not easily seen in dried specimens of the shells, such as are mostly under observation in museums. It is not until these are wetted with spirit that the eyes become conspicuous. Again, Schizochiton, in which they are largest and most evident, is a rarity in museums. A molluscan shell is, moreover, almost the last place in which the naturalist would expect to find eyes, and the Chitonidæ have hitherto in text-books always had the absence of eyes assigned to them as one of the characteristics of their group.

Middendorf[1] named the two distinct layers, of which the shells of Chitonidæ consist, the tegmentum and articulamentum; and Dr. W. B. Carpenter examined the shells of Chitons by means of sections, and observed the perforate structure of the tegmentum in Chiton, writing as follows: " In Chiton the external layer, which seems to be of a delicate fibrous texture but which is of extreme density, is perforated by large canals which pass down obliquely into its substance, without penetrating, however, as far as the middle layer. (Dr. Carpenter has kindly lent me his original sections of Chiton shells, and from what I now know I am able to recognise parts of pigmented eye-capsules in one labelled C h i t o n s p i n i g e r .)"[2] The late Dr. Gray wrote, in his paper on the "Structure of Chitons:" "The greater number of species have a part of the valve which is not covered by the mantle, but exposed. This exposed part consists of a perfectly distinct external coat, p e c u l i a r, I b e l i e v e,

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[1] " Beiträge zu einer Malacozoologia Rossica," 'Mém. de l'Acad. de St. Petersbourgh Sc. Nat.,' Ser. iv, t. vi, 1849.

[2] 'Cyclopædia of Anatomy and Physiology,' article " Shell," p. 565.

to the shells of this family.   The outer coat of these valves is separated from the lower or normal portion by a small space filled by a cellular calcareous deposit, which is easily seen in a section of the valves."[1]   In 1869 Dr. W. Marshall[2] made a great advance in our knowledge. He found that the tegmentum of Chitons was perforated by a series of fine vertical canals, which open at the surface in a series of cup-shaped apertures, and that these vertical canals open into a series of horizontal canals running in the space between the apposed surfaces of the tegmentum and articulamentum, and that these canals opened on the under surface of each shell.   He further found that the larger vertical canals, before reaching the surface, became enlarged and gave off each a crown of smaller canals also terminating at the surface in cup-shaped apertures, and that the canals and apertures, small and large, are distributed evenly over the outer surface of the shell.   He decalcified the shells, and found in the canal system ramifications of soft tissue, which he recognised as offsets of the mantle and considered homologous with those of Balanidæ and Brachiopods. He erroneously regarded the soft tissue ramifications as tubular and respiratory in function.   In 1882 Van Bemmelen, following up his researches, examined the structure of the soft tissues contained in the shell of Chiton marginatus, and discovered that the tegmentum is entirely filled with papilliform bodies which terminate the branches of the network and occupy the surface perforations described by Marshall.   He figures and describes the structure of these papillæ and their relations to the tegmentum, and propounds certain theories as to their homologies which will be referred to in the sequel.   At the time at which I wrote my preliminary account of my discovery of eyes in the shells of the Chitonidæ I was not aware of the existence of Dr. Marshall's and Mr. Van Bemmelen's memoirs, and thought that the papillæ in the tegmentum were also new to science.   I much regret that I should have inad-

[1] J. E. Gray, "On the Structure of Chitons," 'Phil. Trans.,' 1848.

[2] W. Marshall, "Note sur l'histoire Naturelle des Chitons," 'Archives Neerlandaises des Sciences exactes et nat.,' t. iv, 1869.

vertently ignored the claims of these authors to priority in this matter. I am much indebted to Dr. Marshall for having kindly drawn my attention to the two papers. My study of the structure of the shells in numerous genera of the Chitonidæ in connection with my investigation of the structure of the eyes has, however, I believe, thrown much new light on the nature and homologies of the papilliform organs.

METHODS.

My observations have been principally made on vertical and horizontal sections of decalcified shells. In my investigations on the structure of coral, I have had much experience in the decalcification of tissues for the purpose of histological examination. I have tried many methods of slow decalcification recommended, with the result of finding that for all purposes, including the decalcification of the shells of Mollusca, a comparatively rapid decalcification with nitric acid yields the best results. I place the fragments to be softened, which have previously been hardened in strong alcohol, in a vessel holding several ounces of distilled water, and add concentrated nitric acid drop by drop till a brisk ebullition commences, making a three or four per cent. solution. If the decalcification is not completed in twelve hours, I transfer the object into fresh distilled water and add acid as before. I obtain better results by this method than any other.

Structure of the Shells and their Contained Soft Tissue Ramifications.

The tegmenta of the shells of nearly all, if not all, Chitonidæ are perforated at the surface by circular apertures or pores of two sizes, arranged in more or less definite patterns with regard to one another, and sometimes with regard to the eyes also. As the arrangement of these pores must in future become of systematic importance it is convenient to adopt some terms for them, and I shall call them megalopores and micropores. The pores are constantly thus of two sizes, the

difference between the two in size being considerable, and there being no pores of intermediate size between the two. The mouth of each megalopore leads into a cylindrical chamber hollowed out in the thickness of the tegmentum, perpendicular to its surface and more or less dilated in accordance with the form of the papilliform body contained within it. This cylindrical chamber is continued below into a wide canal, which in its course towards the plane of junction of the tegmentum with the articulamentum is curved towards the girdle margin of the tegmentum (Pl. VI, fig. 6, *pp*). On reaching the plane of junction it joins a plexus of wide main canals which ramify horizontally in this plane, parallel with the surface of the tegmentum.

From the sides of the megalopore chambers are given off fine canals, which perforate the tegmentum in a direction vertical to its surface, and join the bases of the micropore cavities. In some species a considerable proportion of the micropore canals are also given off direct from the main vertical branches of the horizontal plexus, as in Corephium aculeatum (see Pl. V, fig. 8). Those springing from the megalopores may be given off from each macropore chamber at the same, or nearly the same, height all round, or at very various heights (see Pl. IV, fig. 10, *bb*).

The tegmentum when decalcified persists as a homogeneous apparently horny substance, which in some species shows a finely fibrous structure (Pl. VI, fig. 4), but in others appears almost structureless. This substance, which is in the recent state of the shell impregnated with the lime salts, is termed by Middendorf the stroma, and by Marshall, Reincke and Van Bemmelen the cuticula. It retains in the decalcified condition both the form and dimensions of the tegmentum itself, and thus in sections of the decalcified shell the disposition of the contained soft structures with regard to the hard parts is clearly displayed.

The plexus of horizontal main canals is occupied in the horny shell by a corresponding ramification of strings of soft tissue, which are offsets of the mantle substance. These

offsets enter the canal plexus by two sets of openings ; firstly,
at the margins of the tegmenta, which adjoin the borders of the
girdle by a series of fine apertures in the shell substance,
which occupy narrow band-like areas intervening between the
tegmenta and upper surfaces of the articulamenta at their lines
of junction with one another.   These bands are sieve-like in
appearance, being perforated all over, and lie just beneath the
external margin of the tegmenta. Secondly, offsets of the
mantle tissue enter the canal plexus at the incisuræ, and by
means of fine pore-like apertures on the under surfaces of the
shells.   These pores may be irregularly scattered, as, e. g. in
the case of the anterior and posterior shells of Corephium
aculeatum, or they may be concentrated along the so-called
sutural lines of the shells which spring from the marginal
notches or incisuræ.   The sutural lines where present in the
anterior and posterior shells radiate from the apices (or
mucrones) of the shells to the marginal notches.   There are
six such radiating sutural lines in the anterior shell of Schi-
zochiton, and six corresponding notches (see Pl. IV, fig. 5.)
On each median shell there are a single pair of lateral sutural
lines, and a corresponding single pair of notches.   The sutural
lines are marked on the under surfaces of the shells by a series
of small slit-like apertures, directed transversely to the lengths
of the lines, and when the shell is removed from its bed corre-
sponding minute transverse processes of the mantle are seen
projecting along corresponding lines on its surface, and torn
across.   Processes of the mantle tissues also enter the shell
canals at the bottom of each marginal notch, and from the
notch longitudinal canals run in the shell substance along the
sutural lines above the series of slit-like apertures.

The strings of soft tissue forming the horizontal plexus show
a finely fibrous structure, and contain numerous nuclei and
fine granular matter. They are not canals as believed by
Marshall.   They contain nerve-fibres within them, as is
certain from the fact that some of them expand into retinas of
typical structure in the eyes.   I have been unable to trace the
nerves supplying the soft tissue ramifications of the tegmenta

directly to their source, but it is probable that they proceed
from the parietal (branchial) nerves. When sections cut
vertically through the decalcified tegmentum, so as to include
the adjacent articulamentum and girdle, the whole shell and
its attachments in situ are examined, I find abundance of
fibrous structures passing from the girdle tissues directly to
join the plexus of tissue in the tegmentum. These series of
fibres are definitely arranged and readily stained, are of deep
origin, and cannot be regarded as mere processes of the mantle.
I have, however, been unable to trace them to any definite
source amongst the muscular tissues. Similar fibres enter the
tegmenta on their under sides abundantly along their sutural
lines. I believe that nerves must accompany these fibres or
form part of them. Haller describes a series of mantle nerves
as given off from the branchio-visceral cords between every
two gills. Each nerve turns outwards towards the mantle
border. He was unable to determine whether it also gives
off fibres which proceed inwards, and supply the body wall
beneath the shells.[1] The nerve-fibres are not to be distin-
guished in the main stems of the ramifications from the tissue
with which they are bound up, but within the eye capsules
the optic nerves break up into bundles of fine fibres which
supply the retina and must be nervous in nature.

Megalæsthetes and Micræsthetes.

From the ramifications of soft tissue are given off branches
to each of the megalopore canals; these follow the curved
course of the latter and expand within the megalopores into
the " papilliform bodies " of Van Bemmelen, to which I shall
apply the name megalæsthetes, believing that they are peculiar
organs of touch and are at all events peculiar to Chitonidæ and
essentially different in structure and origin to the spines borne by
the girdle in that group. They require a special designation.
In some species the strands passing to the megalopores pass

[1] B. Haller, "Die Organisation der Chitonen der Adria," 'Arbeiten aus
dem Zool. Inst. der Universitat Wien,' T. iv, 3 Heft, 1882, S. 10.

directly without branching as separate strings from the plane
of ramification to the megalæsthetes. This is the case in
Acanthopleura spiniger (Pl. VI, fig. 6); in other forms
larger primary branches arise from the ramifications, and, taking
a course vertical to the surface, give off the strands leaving the
megalæsthetes on secondary and tertiary branches (Pl. VI,
fig. 8). The mode of ramification is probably dependent on the
thickness of the tegmentum. The macræsthetes where fully deve-
loped, as, for example, in Acanthopleura spiniger, are more
or less fusiform bodies which occupy the cavities of the mega-
lopores. Externally at the mouths of the pores they terminate
in obconical or somewhat dice-box shaped plugs of transparent
highly refracting tissue, which are extremely conspicuous when
the decalcified tegmentum is viewed from the outer surface
under the microscope. Internally their bodies are directly
continuous with their respective strands of soft tissue (Pl. VI,
fig. 6, *a, p*). The bodies of the megalæsthetes are composed
of a number of cylindrical strands of tissue held closely
together so as to form a bundle which, on transverse section,
shows the component strands cut across without indication of
any definite concentric arrangement, Some of the strands show
a transverse situation, whilst others are not striated. They bear
nuclei at intervals. I have not been able to examine these
structures in living specimens, or such as have been specially
prepared for histological examination, and therefore am uncer-
tain as to the details. Van Bemmelen (l. c., fig. 11) has figured
a megalæsthete of Chiton marginatus, giving histological
details of the body of the organ, which are, I feel sure, more
correct than mine.

The terminal knobs, however, of all the megalæsthetes which
I have examined, except, perhaps, in Chitonellus, show a more
complicated structure than van Bemmelen represents in C.
magnificus. All the terminal knobs terminate in a flat disc.
This disc shows, on careful focussing, a series of concentric rings
(Pl. V, fig. 8, *a*), as if composed of a series of concentric layers or
inverted cones fitted one within the other. Further, the neck of
the inverted cone or dice-box forming the knob shows a series

of transverse ring lines (Pl. VI, fig. 6, *a*) as if composed of a series of superposed discs. Towards the base of the knob these lines instead of being simply transverse become bent towards the body of the megalæsthete as if the knob were there composed of a series of cup-like layers. Between these transverse lines the tissue of the knob is dotted with very fine granules. The knobs of the megalæsthetes appear to be capable of protrusion from the mouths of their pores and retraction, as many were found protruded in spirit specimens. To the organs contained within the micropores I shall give the name micræsthetes. Van Bemmelen's figure, above referred to, shows four micræsthetes as given off from the summit of the body of a megalæsthete of Chiton marginatus.

In Pl. VI, fig. 6, are shown similar micræsthetes supplied by small strands given off from the megalæsthetes in Acanthopleura spiniger. The micræsthetes are small, knob-like bodies, exactly corresponding in structure to the knobs of the macræsthetes. They are similarly obconical in form, and exhibit exactly similar concentric and transverse ring-marks (Pl. V, fig. 8, *b*), and are obviously homologous organs. They are the terminations of fine strands of tissue, which in Acanthopleura spiniger and the genus Chiton appear to be given off only from the sides of the megalæsthetes and from the optic nerves, but in Corephium aculeatum (Pl. V, fig. 8, *b*) spring independently directly from the large vertical branches of the main network.

General Position and External Appearance of the Eyes.

The eyes in the Chitonidæ are entirely restricted to the outer surfaces of the shells on their exposed areas—the surfaces of the tegmenta. They never occur on the laminæ of insertion, the articulameta, nor on the girdle or zone of the mantle which is occupied, as is well known, by various calcareous structures, some of which have been carefully investigated by Reincke.[1]

[1] "Beiträge zur Bildungsgeschichte der Stacheln, &c., im Mantel rande der Chitonen," 'Zeitsch. für wiss. Zool.,' Bd. xviii, S. 305.

On the intermediate or middle shells the eyes are confined to the areæ laterales or to the lines of demarcation between the areæ laterales and the area ventralis, which latter is usually entirely devoid of them. The eyes, which are mostly circular in outline as seen on the shell surfaces, measure about $\frac{1}{175}$ of an inch in diameter, in Schizochiton incisus $\frac{1}{35}$ of an inch, in Acanthopleura spiniger and in Corephium aculeatum, in which they are oval in outline, $\frac{1}{400}$ of an inch by about $\frac{1}{600}$. In Enoplochiton they are smaller still, and only with difficulty seen at all. The eyes appear when viewed by reflected light with a simple lens or low power of the compound microscope as highly refracting convex circular spots, looking as if made of glass or crystal (see Pl. IV, figs. 1, 2, 3, 4). The highly refracting spot, the cornea, is set off by a surrounding narrow zone of dark pigment, which is the margin of the pigmented eye capsule which forms an iris-like structure round the lens, and which is seen through the superficial shell substance (Pl. IV, fig. 3). Through the centre of each cornea is seen a smaller circular area, somewhat darker than the aperture of the pupil, but showing a brilliant spot of totally reflected light due to the lens.

Structure of the Eyes.

The eyes are evidently to be regarded as having arisen as modifications of megalæsthetes. They are connected with the same network of soft tissues as terminal organs of its ramifications in the same manner, and have points of resemblance to them which are convincing as to the homogeny of the two. The soft structures of each eye lie in a more or less pearshaped chamber, excavated in the substance of the tegmentum. The stalk of the pear, which forms the canal for the passage of the optic nerve, is directed always towards the free margin of the tegmentum whence the nerve reaches it. In Acanthopleura the eye chambers and the neural canals continued from them follow in direction the same course as the megalopores and their canals, and join the main canal ramifications in

exactly the same manner (Pl. VI, fig. 6). One side the bulb of the pear, more or less near its extremity, is closely applied to the outer surface of the tegmentum (Pl. VI, figs. 4, 5), and here its wall is pierced by a circular aperture, the pupil-like opening. This opening is covered by the cornea, the periphery of which extends to a considerable distance beyond its margin all round (Pl. VI, fig. 6, f).

The cornea is a concavo-convex, watchglass-shaped lamina. It is calcareous in structure, being continuous all round its margin with the superficial calcareous layer of the tegmentum. It resists the action of strong boiling caustic alkalies, but collapses at once when treated with acid. In sections of the undecalcified tegmentum it shows itself to be formed of a series of concentric lamellæ of transparent hard substance. Probably a continuation of the cuticular substance of the tegmentum is present in its substance, but I have been unable to demonstrate the existence of such by means of acids.

The pear-shaped cavity of the eye in the tegmentum is lined by a dark brown pigmented membrane, of a stiff and apparently somewhat chitinous texture, which forms the eye capsule. This capsular membrane exactly follows the shape of the eye cavity, except near the surface of the tegmentum, where its margin curves inwards beneath the cornea, forming a sort of iris and bounding the circular pupil, which, as before mentioned, is of less diameter than the cornea. The aperture of the pupil is occupied by the front surface of the lens. The lens is perfectly transparent and hyaline and strongly biconvex, it is filled in behind the iris aperture. It is composed of soft tissue and dissolves in strong acetic acid gradually and completely, showing a fibrous distinct structure in the process. In Acanthopleura spiniger the lens is a little flatter in front than behind (Pl. VI, fig. 6, g). There is a space between the front surface of the lens and the cornea.

The optic nerve at some distance from the eye, where arising from the general ramification, is a compact strand completely identical in structure in Acanthopleura, with the strands proceeding to the megalæsthetes. In Onithochiton

the optic nerves are distinguished from the strands supplying the megalæsthetes by being slightly pigmented for a considerable extent of their course. A large proportion of the eyes in Ornithochiton are supplied by nerves which are given off from the soft tissue strands entering the shell along the sutural lines, but many eyes are also certainly supplied by pigmented strands, which can be traced only to the free margins of the tegmenta adjoining the girdle. In those shells in which only single rows of eyes are present coincident with the sutural lines, the eyes seem to be all supplied by strands passing from the sutural line and specially ramifying in order to reach them. Within the pigmented tubular prolongation of the eye capsule the numerous fine fibres composing the optic nerve become separated from one another and loose. Immediately beneath the retina the fibres become still more widely separated, forming an expansion of fibres. The retina is formed on the type of that of Helix, and not, as might have been expected, on that of the dorsal eyes of Onchidium or the eyes of Pecten. The fibres of the optic nerve do not pass in front of the layer of rods to be distributed to them from in front, but are directed to the rods directly from behind. The retina presents a single layer of short but extremely well-defined rods (Pl. VI, figs. 6, 7), the extremities of which are directed towards the light. The rods, when viewed from the surface of the layer they compose, are seen to be hexagonal or pentagonal in outline, and each contains a nucleus. They form a layer which is concave towards the lens, there being a space between the hind surface of the lens and the concave face of the layer.

The rods closely resemble in appearance those figured by Semper as occurring in Onchidium. Immediately beneath the rod layer is a stratum or several layers of nuclei amongst the ramifications of the nerve-fibres. The structure of the retina, as described, has only been made out in specimens of Acanthopleura spiniger, which alone of the material available were in a condition of preservation sufficient to permit it. Similar expansions of the optic nerve have been seen, however, to occur in many other forms.

The only pigment present in the eyes examined is that by which the eye capsules are rendered opaque.   No pigment seen in connection with the rods or in connection with the nervous elements.   Possibly the absence of such pigment is due to the imperfect preservation of the material.

Not all the fibres of the strand entering the eye cavity proceed to the retina.   A large number of peripherally placed fibres pass outside the retina all round, and, passing through apertures in the iris at its outer margin, end at the surface of the shell all round the area occupied by the corneæ.   They terminate in micræsthetes exactly corresponding in structure to the other micræsthetes present and identical with them in structure.

They apparently form a sensitive zone round each eye, and their strands arise from the optic nerve just as do those of many of the other micræesthetes from the megalæsthetes (see Pl. VI, fig. 6, b' b'; fig. 4, b; Pl. V, fig. 8, b' b').   On their way to the surface these strands, given off by the optic nerves to the micræsthetes, traverse a series of slit-like perforations of the ris, which are conspicuous, and at first very puzzling features in the iris structure when the eyes are viewed in the decal-cified tegmentum from its external surface by transmitted light (Pl. VI, fig. 5, b b).

In the eyes of some forms when thus viewed, an open fold or gutter leading from the bulb superficially along the stalk of the pear is seen, curiously recalling the choroid fissure (Pl. VI, fig. 5).   The occurrence of double eyes, combinations of two eyes fixed closely side by side with a common nerve stalk, is not an uncommon mode of growth.

Growth of the Tegmentum, Eyes and Æsthetes.

The tegmenta increase in growth by additions formed at their margins where they adjoin the girdle regions of the mantles.   The additions are probably made by the mantle tissues which immediately abut on the tegmental margins.   At the basal margins of the tegmenta of all forms in which

eyes occur in any numbers, the eyes may be seen in all stages of formation. The eyes are formed in the position which they always occupy when complete, namely, with the stalk of the pear-shaped pigmented capsule containing the optic nerve turned towards the margin of the tegmentum adjoining the girdle, and the bulb of the eye directed towards the shell apex. The first trace of a developing eye is a semilunar fold of pigmented eye-capsule. This increases till it becomes horse-shoe shape with the pupil margin well defined. Next the lens appears, and the cornea and traces of the nervous elements, and the nerve capsule gradually becomes longer, and finally the narrow canal into which it contracts is added. At each successive stage it appears like a segment of a complete eye, the tail so to speak of which has been cut off transversely, less and less shortly.

The megalæsthetes are similarly formed as the tegmenta increase in growth at their free margins. By preparations so made as to show the junction of the margin of a tegmentum with the girdle, the megalæsthetes may be seen in all stages of formation in a similar manner to the eyes. There is no indication of any enclosure of the spines borne by the girdle within the substance of the tegmentum in course of its formation, and there are no traces of any bodies resembling the megalæsthetes or micresthetes in the girdle tissues; none such ever occur beyond the actual margins of the tegmenta.

Presence or Absence of Eyes in Various Genera of Chitonidæ, differences in the arrangement of the Eyes when present, &c.

In some genera of Chitonidæ eyes are entirely absent. This is the case with the genus Chiton, which has, as shown by Marshall and van Bemmelen, the usual megalopores and micropores, megalæsthetes and micræsthetes, but in no species of which I have been able to detect any trace of eyes. Van Bemmelen investigated Chiton marginatus, and I especially by decalcification only C. magnificus and C. marmo·

ratus; but the eyes in the shells of the Chitonidæ may, by a little practice, be readily detected by examining the dried shells directly with a hand lens; and I have examined rapidly in this way all the likely looking specimens in the extensive collection in the British Museum, and that at Montreal, and feel pretty certain that no eyes will be found in the genus Chiton, as now distinguished there. In Molpalia, Maugina, Lorica, and Ischnochiton, there are apparently no eyes as far as a cursory examination has yielded evidence to me. In Chitonellus there are certainly no eyes.

The arrangement and the forms of the eyes vary considerably in different genera, and these characteristics will probably prove of considerable value in the classification of the Chitonidæ, which has hitherto proved so difficult a problem.

The genus Schizochiton is distinguished by having the mantle deeply notched posteriorly, in correspondence with a deep median notch in the hinder border of the posterior shell (Pl. IV, fig. 1, c). In Schizochiton incisus the eyes are restricted to single rows traversing the sutural lines. There are six rows of eyes on the anterior shell, corresponding with the number of marginal notches; two on each of the middle shells, and six on the posterior shell—twenty-four rows altogether, with an average of about fifteen eyes in each, or in all 360 eyes (see Pl. IV, figs. 1, 2, 3, 4, 5). In the single specimen carefully examined all the rows except one have the eyes arranged in a single straight row at regular intervals, but at the base of one row there are as an exception two eyes side by side. There are also in one or two places a very few irregularly scattered eyes on the areæ laterales, showing that the condition here existing has probably been derived from an ancestral one in which the eyes were not concentrated into lines, but more widely diffused on the shell surface. In one row again, one eye is missing from the spot on which it ought to occur (Pl. IV, fig. 2). The rows of eyes are placed on raised ridges on the shell surface, formed by the development of tubercles on the prominent ridges with which the surfaces of the tegmenta are ornamented. The eyes in Schizochiton are the largest I have

found in any of the Chitonidæ, measuring $\frac{1}{175}$th of an inch in diameter. When seen under the microscope, either by reflected light or by transmitted light in thin ground sections of the tegmentum, they are extremely brilliant and conspicuous.

In Acanthopleura spiniger (see Pl. VI, figs. 1, 2, 3, 6) the eyes are irregularly scattered around the bases of the tubercles with which the surface of the tegmentum is covered, and are confined, in the specimens I have examined, to the region of the margins of the tegmenta adjoining the girdle. The eyes of this species seem to be liable to be broken or to flake off in consequence of the decay of the surface laminæ of the tegmentum. Hence those remaining on old specimens are probably those most recently formed by the mantle at the margin of the tegmentum. The process of the formation of eyes pari passu with the growth of the shell has been already described. In some specimens apparently, according to the existing systematic rules to be referred to the species Acanthopleura spiniger, I have been able to find no eyes at all. It will be necessary to examine a series of specimens of various ages to discover whether the eyes are originally more widely extended over the shell surface in the young or always marginal, and thus of late appearance in the life of each individual in this species.

In Acanthopleura spiniger there are large, prominent rounded tubercles on the shell surface; possibly they act as fenders to preserve the eyes which lie around their bases from attrition. The micropores and megalopores are borne on isolated, ovoid prominences of the tegmentary surface; each prominence bears a single megalopore on its summit, surrounded by a zone of micropores (Pl. VI, fig. 3).

In Acanthopleura piceus (Pl. VI, figs. 8 and 9) there are somewhat similar tubercles to those occurring in A. spiniger, but they show a tendency to form ridges. The eyes are, as in A. spiniger, marginal in position, but more numerous.

In a large Corephium aculeatum, the tegmenta of which were densely covered by a green alga, which perforates and penetrates the shell substance, immense numbers of eyes were

found when the alga was scrubbed off, and at the most recently formed margins of the tegmenta not yet encroached upon by the plant (Pl. VI, figs. 10, 11, 12).

The eyes are very small and their corneæ are oval in outline, the long axes of the ovals being directed vertically in the direction to the heights of the shells.   The eye-capsules reach to only a small depth in the thickness of the tegmenta.   The megalopores and micropores are disposed in vertical parallel lines with great regularity, the megalopores occurring at regular intervals in the lines of micropores (Pl. VI, fig. 11).   A considerable proportion of the micræsthetes are borne on strands independent of the megalæsthetes.   The tegmentary surface is covered with rows of tubercles, so disposed as to form regular series radiating from the apex of each shell, and also corresponding with one another in position horizontally.   The eyes are never placed on the tubercles, but lie on the flat surface of the tegmentum between them, and it is possibly because of the existence of the tubercles all over the tegmentary surface that the eyes do not get entirely obliterated in the older regions of the shell.

The eyes are present in enormous numbers.   I estimate roughly the numbers present on the anterior shell alone at 3000, counting only the younger ones, which are in good condition, near the free margins of the tegmentum, and not the older more or less destroyed by the boring of the shell by algæ and animals on the rest of the areæ.   On the remaining shells, at a moderate estimate, reckoning as before only the eyes in tolerable condition, there must be at least 8500 eyes.

In Enoplochiton niger the eyes are excessively minute, and would not have been recognised at all as such had not the larger eyes in other forms been previously studied.   They are here also confined to the margins of the tegmenta (Pl. IV, figs. 6, 7, 8, 9).

The cornea is slightly oval, as in Corephium aculeatum, and as in that species the megalopores and micropores are disposed in vertical lines.

In Tonicia marmorata the eyes have the peculiarity of

being sunk in little pit-like depressions of the shell surface (Pl. V, figs. 1, 2, 3). This no doubt is a contrivance for preventing them from being worn off, and the result is that they are all retained complete up to the apices of the shells in large old specimens. They are arranged in single straight rows, radiating from the apices on the anterior and posterior shells, disposed with considerable symmetry. There are thirty-four such radial lines on the anterior shell in one specimen containing about eighteen eyes each. On each lateral area of the intermediate shells there are from two to four similar rows of eyes, with a few additional eyes also grouped irregularly. In some forms placed in the genus Tonicia, in the British Museum collection, there are no eyes present. It probably will be found that these should be placed in a separate genus.

I have been unable to obtain any specimen of any species of Tonicia preserved in spirits for examination of the soft tissues of the eyes. The pores are arranged in vertical rows, as in Corephium.

In Ornithochiton the eyes are not sunk so deeply in pits as in Tonicia, but are disposed somewhat as in that genus, though the rows are not so regular (Pl. V, figs. 4, 5, 6, 7); the pores, megalæsthetes and micræsthetes are arranged as in Tonicia. The numerous eyes on the terminal shells are disposed in the radial rows at tolerably regular intervals, so as to form transverse rows also parallel with the tegmental margins. Amongst these transverse rows some occur at intervals which are characterised by the eyes composing them being much smaller than the average size.

In Chitonellus there are no eyes, and the æsthetes are apparently in a primitive condition of development. They are, as elsewhere, confined to the tegmenta, and in these areas so small in this genus, are not numerous. I have not had any very well preserved material to work on, but there appear to be both micræsthetes and megalæsthetes present. These terminate in the typical obconical knobs, but their bodies appear to be almost undeveloped. They bear no resemblance to the calcareous spines of the girdle.

General Remarks.

I regard the megalæsthetes and micræsthetes as probably organs of touch which may to some extent take the place of the tentacles which are absent in the Chitonidæ. I base my conjecture as to their having a sensitive function on the fact that the megalæsthetes are in certain genera of the Chitonidæ converted into undoubted organs of special sense, viz. eyes. It is important that experiments should be made on living Chitons to determine whether the æsthetes are protrusible and are used as organs of touch, and also as to the sensitiveness to light of the eyes. I have searched in vain for any traces of eyes like those of the Chitonidæ in the shells of Patella and allied genera. I am inclined to believe that the megalæsthetes and micræsthetes are not, as van Bemmelen concludes, homologous with the spines of the girdle or rather with the funicles by which these spines are supported.[1] The structure of the megalæsthetes seems to me to be quite peculiar and distinct. The funicles of the girdle spines never give off a series of small offsets like the micræsthetes. The eyes are obviously homologous with the megalæsthetes, yet in none of the Chitonidæ is there a trace of an eye or part of an eye in the girdle region beyond the margin of the tegmentum. In the small plates of shell developed on the girdle in the Chitonidæ and other genera, there are never any megalopores or microspores, or any traces of megalesthetes or micresthetes.

The structure of the girdle contrasts most markedly with that of the tegmentum, and there is an absolutely sharp line of demarcation between the two at the place where they are in contact. This is well to be seen in Onithochiton. In the shell are seen the megalæsthetes and micræsthetes arranged with exact regularity and the eyes extending up to the very margin where some of both are seen, as yet only half formed, whilst in contact with these half-formed growths is the marginal line of the girdle devoid of micræsthetes and mega-

[1] Van Bemmelen, l. c., p. 94, 95. A. W. Hubrecht, "Morphology of the Amphineura," 'Quart. Journ. Micro. Sci.,' vol. xxii, 1882, p. 214.

læsthetes and eyes, but covered by large spines irregularly disposed. Moreover, the peculiar mode of formation of the æsthetes and eyes at the margin only of the tegmentum is evidence against the homology. Were the megalæsthetes homologous with the funicles of the spines, it would be probable that in the growth of the tegmentum funicle-like organs contained in the margin of the girdle would become encroached upon by the tegmentum and included within it to become æsthetes, but such is not the case. Eyes being absent in the Solenogastres, I would suggest that the æsthetes are organs developed originally in connection with the shells in the Chitonidæ, still little differentiated in Chitonellus, and not homologous with the spine-bearing funicles at all, which are of more ancient origin, occurring in Proneomenia. As a comparatively late modification, some of the megalæsthetes have been modified into eyes in certain genera, whilst in Chiton and other forms, the more primitive condition in which they all remain as organs of touch has been retained.

The forms of the Chitonidæ which bear well-developed eyes appear to be mostly non-European. It is therefore not easy to obtain specimens properly prepared for examining the minute structure of the retina in a satisfactory manner, but my father-in-law, Dr. Gwyn Jeffreys, has pointed out to me that Costa[1] figures what are evidently eyes on one of the intermediate shells of a very small species of Chiton, called by him C. rubicundus (Ornithochiton?), which species is common in Sicily. The eyes are figured as mere black dots and referred to as fine punctuations, but are evidently eyes. Possibly some interesting results might be got by examining them in the fresh condition.

In conclusion, I would express my best thanks to Dr. Günther for giving me every facility in making use of the fine series of Chitonidæ in the British Museum, and allowing me to dissect some duplicate specimens preserved in spirits. Also to Professor Westwood, who supplied me with others out of the Hope collection, and to Mr. W. H. Dall, who showed me

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[1] "Fauna di Napoli," 'Animali Molli Chitone,' taf. iii, fig. 1, e.

the Smithsonian collection at Washington and gave me some specimens from the Pacific coast.

Dr. Woodward kindly went over the fossil Chitons with me, but we could not detect any traces of eyes in them.

DESCRIPTION OF PLATES IV, V, & VI,

Illustrating Professor H. N. Moseley's paper " On the Presence of Eyes in the Shells of Certain Chitonidæ and on the Structure of these Organs."

N.B.—All the drawings, with the exception of fig. 2, Pl. IV, figs. 4, 5, and 6, Pl. V, and fig. 8, Pl. VI, drawn by the author, are made from the actual shells by a professional artist, Mr. W. H. Hill.

PLATE IV.

Fig. 1.—View of a specimen of Schizochiton incisus, preserved in spirits, and with the outline of the margin of the mantle somewhat distorted in consequence. a. Anterior shell, with six rows of eyes. b. Posterior shell, with six rows of eyes. c. Anal notch in the posterior shell. d. One of the middle shells, with two rows of eyes. e. Another of the middle shells, which bears as an abnormality three rows of eyes, two on one side and one on the other.

Fig. 2.—One of the rows of eyes of a middle shell of Schizochiton incisus much further enlarged.

Fig. 3.—A single eye from the same specimen as the above, still more highly magnified. b. Calcareous cornea. g. Lens, bordered by the apertures of the iris seen through the cornea. c. Pigmented eye-capsule, seen partly through the superficial layers of the general shell substance.

Fig. 4.—The anterior shell of Schizochiton incisus, outer view ; one half indicated in outline only. On the finished half three rows of eyes are seen borne on raised ridges of the shell.

Fig. 5.—The same shell ; inner view. a a a. Incisuræ marginalis. b b. The sutural lines of pores for the passage of nerves, continued from the incisuræ.

Fig. 6.—Part of a specimen of Enoplochiton niger, viewed from the side. Only a part of one of the middle shells and of the girdle is

shaded. A lower part of the lateral face of one of the middle shells is covered by minute eye specks. *b b.* Calcareous plates of the girdle.

Fig. 7.—Outline of the entire specimen of Enoplochiton incisus of life size, viewed from the side. *a.* Area included by a dotted line, showing the situation of the part magnified in Fig. 6.

Fig. 8.—A small portion of the surface marked *a* in Fig. 6, more highly magnified. *d.* Eyes. *a.* Megalopores. *c.* Area, still more highly magnified in Fig. 9.

Fig. 9.—Area marked *c* in Fig. 8, further enlarged. *a.* Megalopores. *b.* Micropores.

Fig. 10.—Schematic representation of the form and arrangement of the organs of touch in the shell of Chiton magnificus, as seen after decalcification, in a section vertical to the shell surface. *a.* Megalæsthetes. *b.* Micræsthetes. *d.* Stem of a megalæsthetes. *e e.* Main soft tissue strands.

PLATE V.

Fig. 1.—Anterior shell of Tonicia elegans; external view, showing the arrangement of the eyes in radiating lines.

Fig. 2.—Enlarged view of two rows of eyes from the above, only partially shaded. The eyes are sunk in a series of slight depressions, forming a partial groove on the surface of the shell.

Fig. 3.—Portion of the surface of the same, still more enlarged. *a.* Megalopores. *b.* Micropores.

Fig. 4.—Lateral view of one of the middle cells of a species of Onithochiton. The tegmentum has a series of eyes upon it, which commences as a single row superiorly and broadens out into a scattered group inferiorly. *t.* Tegmentum. *a.* Articulamentum. *b.* Incisuræ marginalis.

Fig. 5.—Part of the surface of the same, bearing the scattered eyes much enlarged. *a.* Macropores. *d.* Eye. *c.* Area, shown still more enlarged in Fig. 6.

Fig. 6.—*a.* Megalopore. *b.* Micropore.

Fig. 7.—Anterior shell of the same species of Onithochiton partially shaded, showing the more or less regular radial rows of eyes.

Fig. 8.—Schematic representation of the tactile organs and an eye of Corephium aculeatum, as seen in a section vertical to the surface in decalcified specimens, excepting that the calcareous cornea is here retained. *a.* Free end of a megalæsthete, projecting at the shell surface. *b.* Micræsthete. *b' b'.* Micræsthetes supplied by offsets of the optic nerve, which perforate the iris to reach the surface. *d.* Base of megalæsthete. *e.* Main nerve branch. *k.* Pigmented eye-capsule and cavity of eye-capsule. *n.* Optic nerve. *h.* Iris. *f.* Calcareous cornea.

PLATE VI.

FIG. 1.—Sketch of a lateral view of a specimen of Acanthopleura spiniger. *x.* Small area on the side of the tegmentum of one of the middle shells, which is shown more highly magnified in Fig. 2.

FIG. 2.—The area indicated in the foregoing figure enlarged 65 diameters. *a a.* Prominent rounded tubercles on the shell surface. *b b.* Pore-hillocks, each with a megalopore visible at its summit. *d d.* Eyes.

FIG. 3.—Three of the pore-hillocks of Acanthopleura spiniger shown in the preceding figure more highly magnified. *a.* Megalopore. *b.* Micropore.

FIG. 4.—View of the surface layer of the soft tissues of a decalcified middle shell of a species of Acanthopleura from China, viewed by transmitted light, showing the grouping of the micræsthetes and megalæsthetes in relation with an eye. The cuticula of the tegmentum is seen to be finely fibrous. *n.* Channel, containing the optic nerve. *l.* Lens of the eye. *ch.* Pigmented eye-capsule, forming an iris-like border around the lens. *b.* Tips of micræsthetes, which pierce the iris and terminate at the free surface of the shell around the margin of the cornea. *a a a.* Macræsthetes in chambers, hollowed out in the tissue and continued in the direction of the margin of the shell which adjoins the girdle into nerve canals.

FIG. 5.—A single eye of Schizochiton decalcified. *l.* Lens. *ch.* Pigmented eye-capsule, continued inwards to form an iris. *b b.* Slit-like apertures in the iris, giving passage to the branches given off by the optic nerve to the ocular micræsthetes. *s.* Cleft in the outer wall of the channel for the optic nerve.

FIG. 6.—Schematic representation of the structure of the soft and some of the hard parts in the tegmentum of a shell of Acanthopleura spiniger, as seen in a section vertical to the surface and with the margin of the shell bordering on the girdle lying in the direction of the left side of the drawing. *f.* Calcareous cornea. *h.* Iris. *g.* Lens. *k.* Pigmented capsule of eye. *n.* Optic nerve. *r.* Rods of retina. *n'.* Branches of the optic nerve, perforating the capsule wall and terminating in *b' b' b'.* Ocular micræsthetes. *p p.* Nerves to macræsthetes. *m.* Body of macræsthete cut across. *o e.* Fusiform body of macræsthete entire. *a.* Obconical termination of macræsthete. *e.* Nerve given off by macræsthete to micræsthete *b''.*

FIG. 7.—Rods of the retina of Acanthopleura spiniger, viewed from above in a horizontal section of the eye.

FIG. 8.—Sketch of a specimen of Acanthopleura piceus, viewed from the side, of natural size. *x.* Area on the side of the tegmentum of one of the middle shells, which is seen highly magnified in Fig. 9.

FIG. 9.—The area indicated in Fig. 8, enlarged 26 diameters. The lateral

margin of the shell is indented at intervals; it overhangs the girdle part of which is shown beneath it. *a a a.* Tubercles on the shell surface. *d d.* Eyes.

Fig. 10.—View of the anterior shell of a specimen of Corephium aculeatum, enlarged 2 diameters, showing the regular rows of tubercles. *a.* Articulamentum. *t.* Tegmentum.

Fig. 11.—A portion of the surface of the tegmentum of the same shell near its articulamental margin, enlarged 17 diameters. *a a.* Tubercles forming rows. *d d.* Eyes. *x.* Small area, more highly magnified in Fig. 12.

Fig. 12.—Small area on the surface of the tegmentum of the anterior shell of Corephium aculeatum, enlarged 185 diameters. *d.* Oval eye. *a a a.* Megalopores. *b b b.* Micropores.
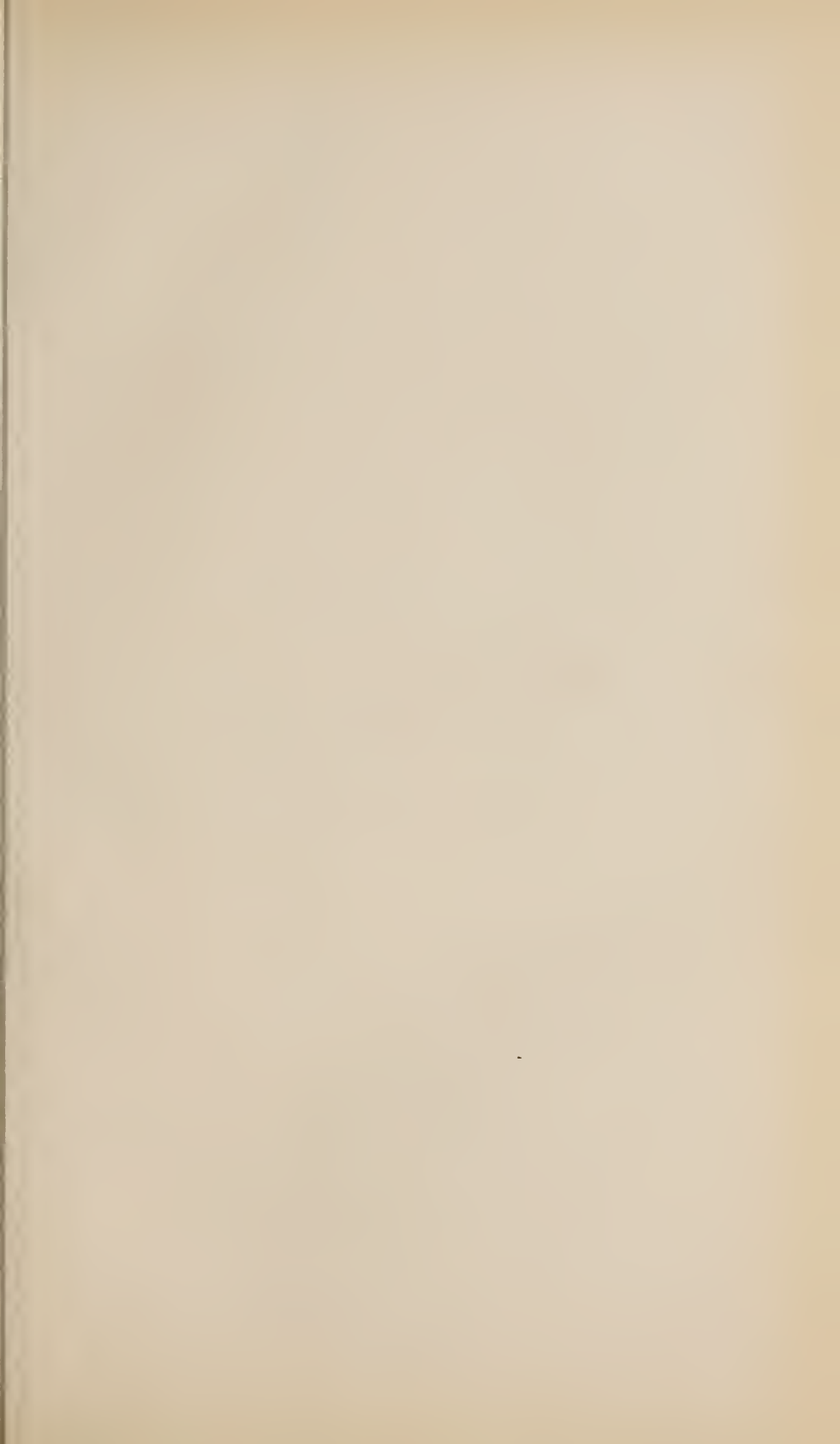
Fig 1 × 3
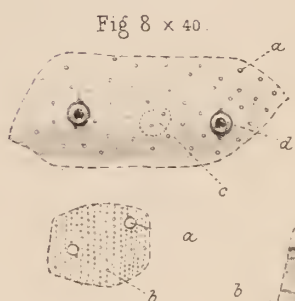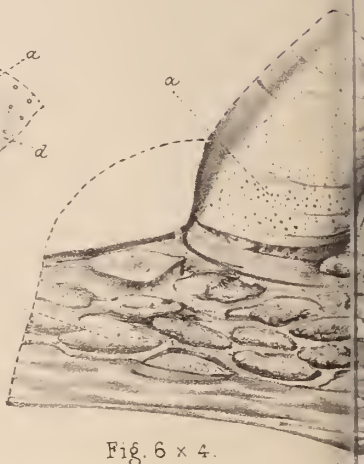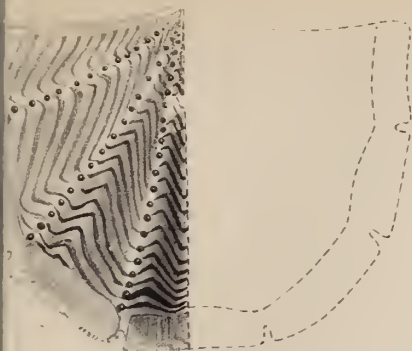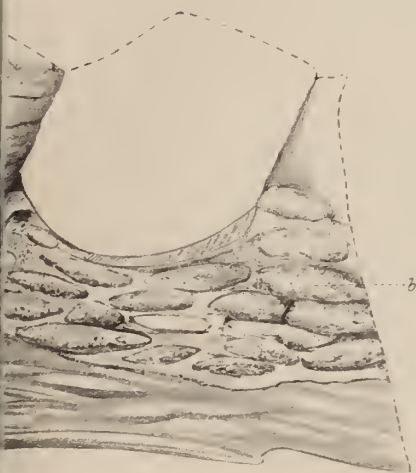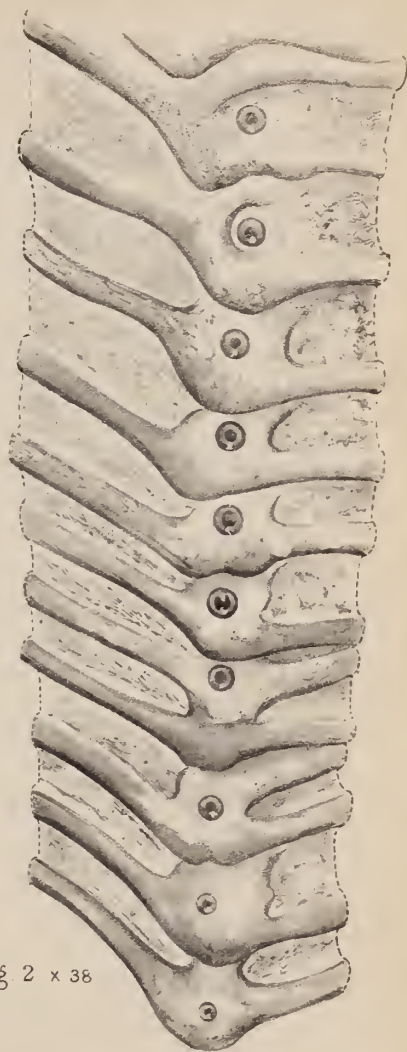
Fig. 5 × 10

Fig 8 × 40.

Fig 9 × 130.

Fig. 6 × 4.

Fig 3 × 20

Fig 4 × 10   *a*

Fig. 7

Fig 2 × 38

Fig. 10.

Fig. 1 × 6.

Fig. 2 × 18

Fig. 3.

Fig

W. H. Hill & E. N. M. del.

Fig. 7 × 14.

Fig 6 × 145.

Fig. 5 × 75.

Fig. 4 × 10.

Fig. 1 nat. size.

Fig. 2 × 65

Fig. 10 ×

Fig. 11 × 17

Fig. 4.

Fig. 12 × 185.

Fig. 5.

Fig. 7.

Fig. 3.

W. H. Hill & H. N. M. del.
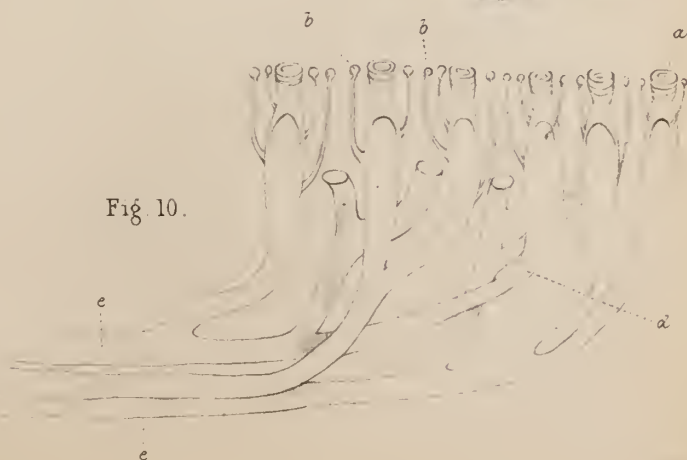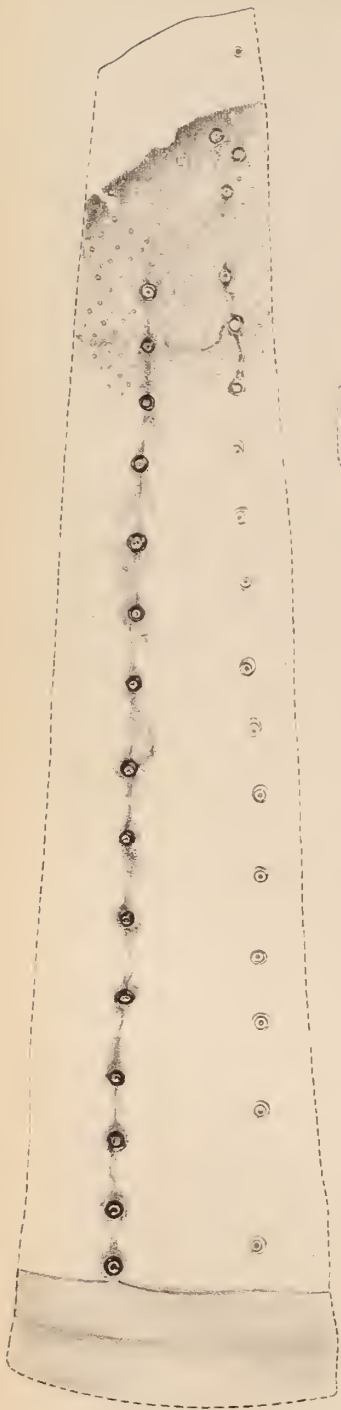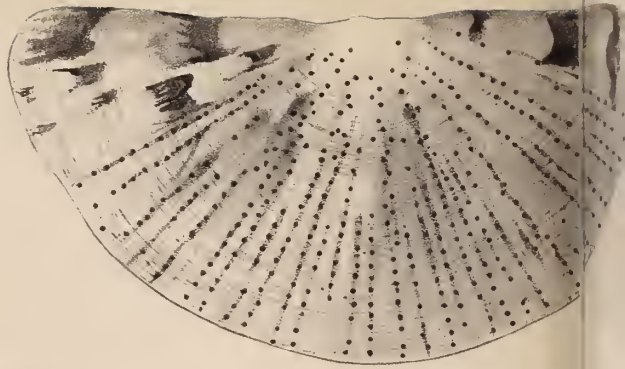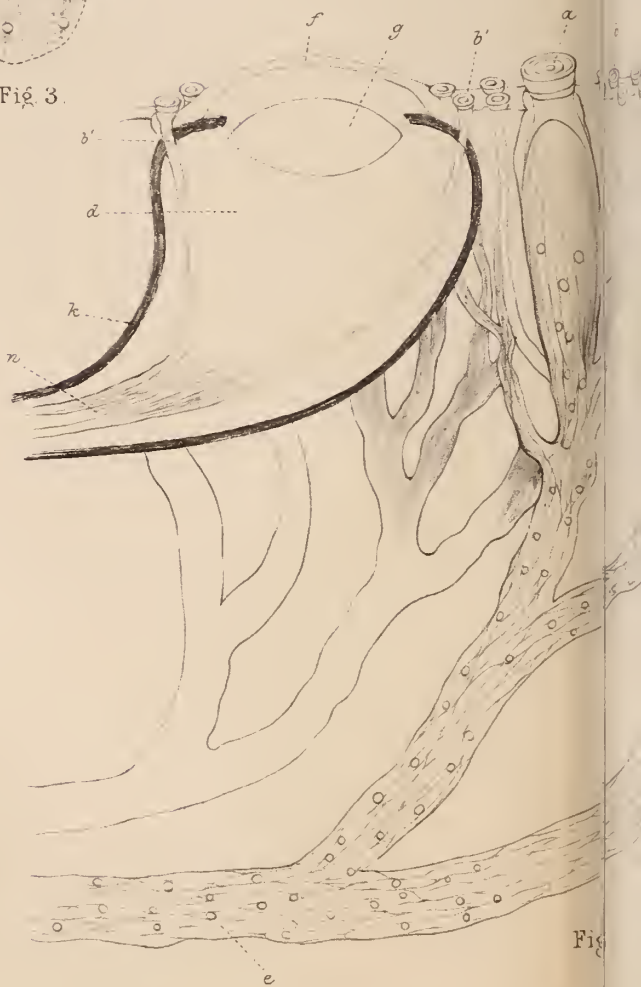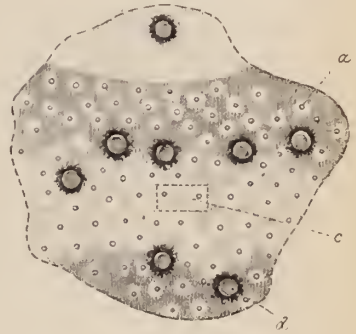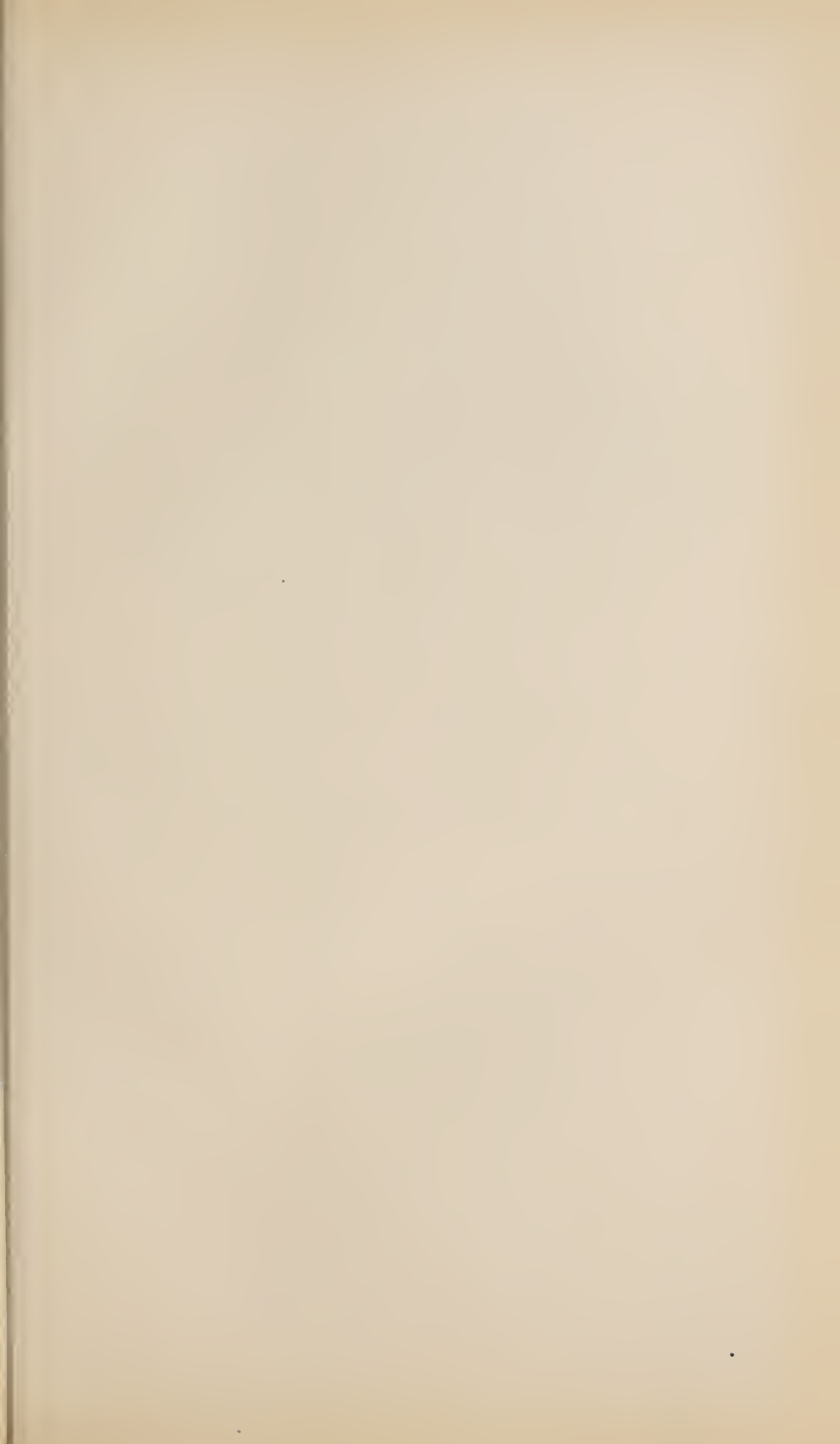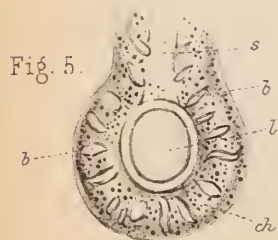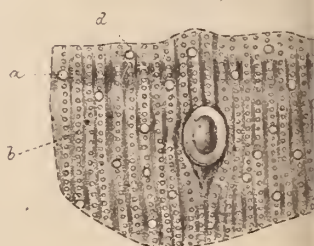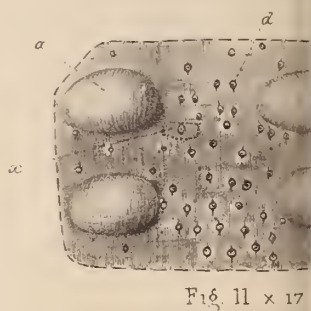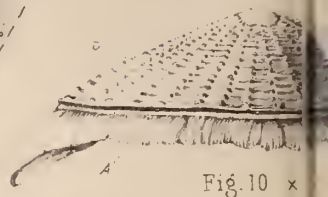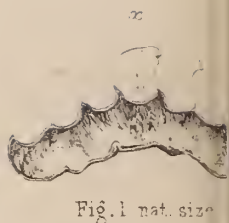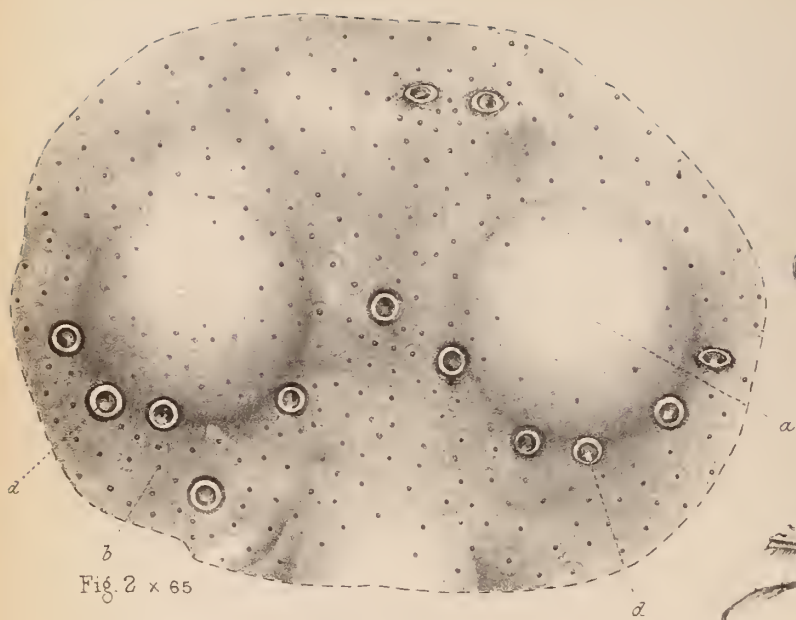
Fig 9 × 26.
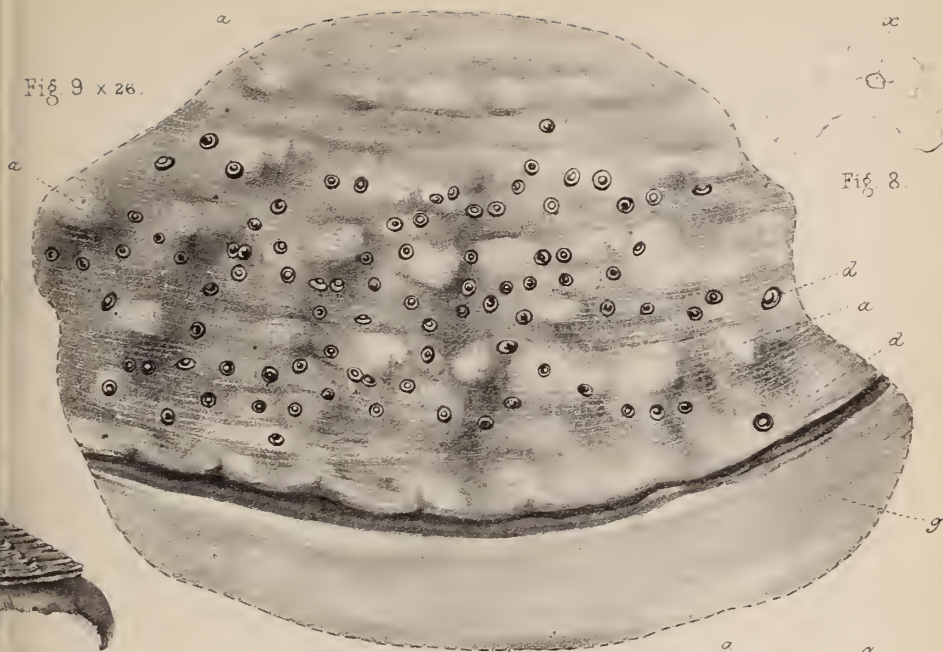
Fig. 8.
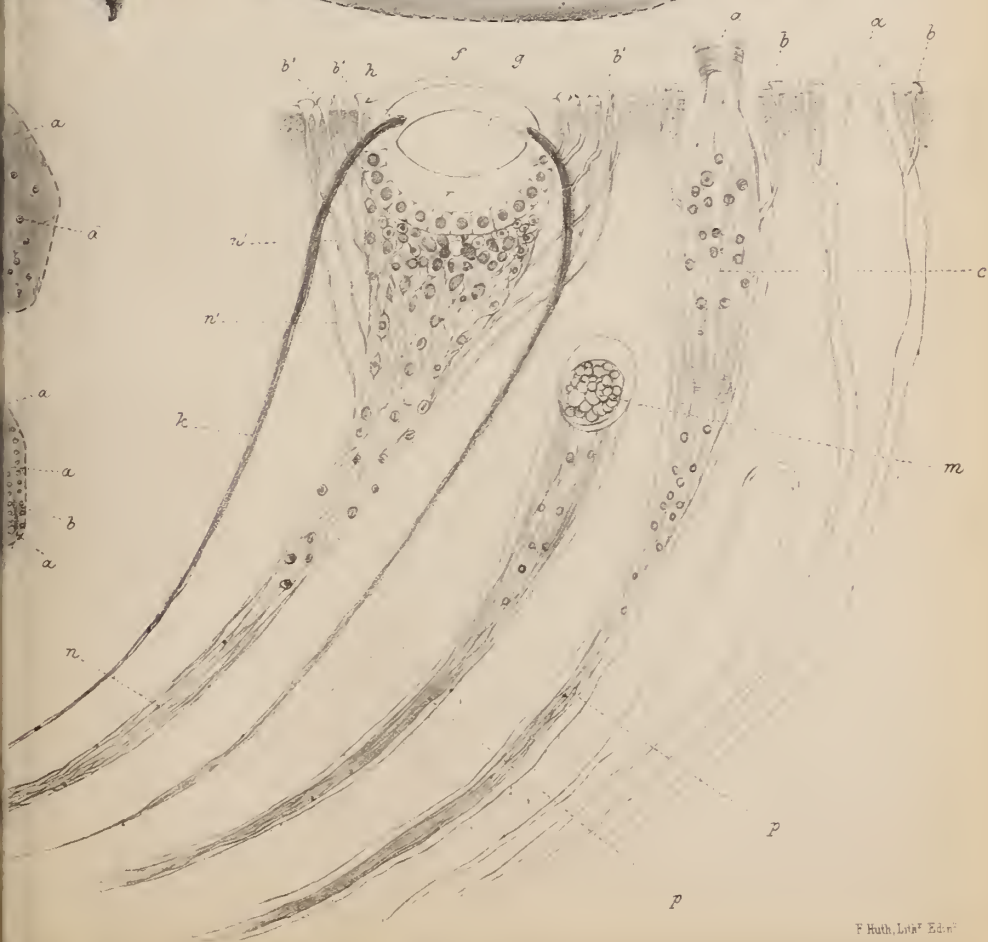
Fig. 6.

Archerina Boltoni, nov. gen. et sp., a Chlorophyllogenous Protozoon, allied to Vampyrella, Cienk.

By

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With Plate VII.

DURING the months of June and July, 1884, I received from Mr. Thomas Bolton, of Birmingham, several gatherings from ponds in his neighbourhood which contained an abundance of a minute, green-coloured, Heliozoon-like organism, to which he directed my attention. Careful study of the material forwarded to me by Mr. Bolton established the fact that the little Protozoon was hitherto undescribed, and that it presented many features of considerable interest.

The discovery of this form is due to Mr. Bolton, to whom English naturalists are deeply indebted for constant supplies of the most interesting and important of our known freshwater micro-fauna, as well as for the discovery of such novelties as the Rhizopod, Lithamœba discus, the Chætopod, Haplobranchus œstuarinus, several new Naidinæ, and not a few Rotifera.

I propose to place the organism thus brought to light in a new genus, which I dedicate to my friend and former colleague, Mr. William Archer, of Dublin (the discoverer of so many Heliozoa), as "Archerina;" and as a specific name I associate with this interesting form that of its discoverer. It will thus stand as Archerina Boltoni.

Occurrence.—Archerina occurs in great numbers in pond-water associated with Desmids and other minute chlorophyll-bearing algæ. Its spherical chlorophyll-corpuscles may at first be mistaken for those of such microscopic plants; but a little attention is sufficient to enable one to detect around many of the bright-green spheres or groups of spheres a halo of radiant protoplasm, frequently in the form of very long and stiff filaments. Once recognised, it is not difficult to distinguish Archerina in its various phases of growth and multiplication from its associates.

Structure and Life-history.—1. Actinophryd-form, fig. 7. —The abundance in which Archerina occurs in the material sent to me by Mr. Bolton has enabled me to trace it in several phases of growth. The most convenient of these to commence with is that represented in Pl. VII, figs. 7 and 13. We have here a spherical body $\frac{1}{2000}$th of an inch in diameter, consisting of a sharply outlined mass of refringent protoplasm, from the surface of which radiate a number of very delicate but stiff filaments, some of them four times as long as the diameter of the sphere and tapering from the base towards the extremity. There are in such a specimen about fifty of these filamentous "pseudopodia," more or less. The base of each filament is relatively broad, and appears to join without penetrating the surface of the sphere. In such specimens I could detect no membrane or pellicle on the surface of the sphere or its pseudopodia. The pseudopodia are motionless, and did not exhibit any streaming of granules such as is seen in Actinosphærium. Within the spherical body is usually one large spherical vacuole (fig. 7), but sometimes there are more, and they may be of various sizes (figs. 8 to 12). Sometimes the whole of the protoplasm of the spherical body appears of a bright green colour (fig. 8), but on causing the organism to roll over one finds that the green colour is limited to two masses, which may be united on one face of the sphere though separated more deeply.

Usually the green colour, in the particular phase of Archerina now under description, appears in the form of two

oval masses lodged in the protoplasm of the sphere, as shown in figs. 7, 10, 11, and 13.

Rarely the green colour is seen to be confined to a single spherical corpuscle (fig. 12) of half the diameter of the whole sphere.

The green colouring matter is chlorophyll, identical in tint with that of the Desmids, Closterium, and Pediastrum, which abound side by side with Archerina. The chlorophyll in Archerina is confined to a definite " chlorophyll-corpuscle," a dense portion of the constituent protoplasm of the spherical body; in fig. 12 this chlorophyll-corpuscle is in a quiescent condition; in figs 7, 8, 9, 10, 11, 13 it is in process of multiplication by division.

No other structural elements can be detected in Archerina in this phase of its growth either before or after the use of re-agents. It consists simply of a sphere of dense protoplasm with radiating pseudopodia, one or more large vacuoles, and a single or bifid chlorophyll-corpuscle. No nucleus can be detected in Archerina. The examination for a nucleus was carefully made. The chlorophyll of the chlorophyll-corpuscle was extracted by alcohol, and the Archerinæ were then stained with borax-carmine; others after similar extraction were stained with Kleinenberg's hæmatoxylin, others with picro-carmine, and some with anilin blue (aqueous solution). In none of these cases could any nucleus or nucleus-like structure be detected, excepting the chlorophyll-corpuscle itself, which (as is usual with chlorophyll-corpuscles) after the discharge of the chlorophyll exhibited a marked superiority over the sur-rounding protoplasm in taking up the staining agent in each of the above instances.

The chlorophyll-corpuscle of Archerina, like those of Spon-gilla and Hydra (and like those of higher plants), appears to consist of modified protoplasm resembling that of a cell-nucleus. In this particular instance it appears that the chlorophyll-corpuscle is actually taking the place of a nucleus. As will be seen directly, the life and growth of the Archerina centres round its chlorophyll-corpuscle. The

division of the chlorophyll-corpuscle precedes and is invariably followed, sooner or later, by the division of the protoplasm of the whole organism.

At the same time it does not appear that there is any ground for regarding the green-coloured corpuscles of Archerina as ordinary cell-nuclei (or rather, one should say, Protozoon cell-nuclei) coloured green by chlorophyll. They have none of the distinctive characters of cell-nuclei as distinguished from chlorophyll-corpuscles. They do not exhibit any differentiation of chromatin substance into fibrillæ or loops at the period of division, and moreover they appear as a rule to divide not into two but into four.

I am not sure that the complete division into two does not occur in the large individuals such as are drawn in figs. 7 and 13; but it is quite certain that in some of these large forms (figs. 14 and 15), and in all the smaller phases of Archerina (figs. 20, 21, 22, 24), the dividing chlorophyll-corpuscle forms a tetrad. It is possible that the curious form presented by the chlorophyll-corpuscles in figs. 7 to 13—when there is an appearance of two oval bodies which are joined by a superficial shell of green-coloured substance on one hemisphere of the organism—may be only preliminary to the breaking up of the chlorophyll-corpuscle into four, and may not really indicate, as it seems to do, a division into two.

There are reasons for regarding these larger forms as exceptional, inasmuch as they appear to have emerged but recently from the encysted condition (figs. 1, 2, 3, 4, 5, 6). When the Archerina has once fairly started on an active vegetative growth (as in the groups of smaller individuals) there is no doubt that the division of the chlorophyll-corpuscle usually and characteristically proceeds by the simultaneous fission of the corpuscle into four segments (fig. 25), and consequently produces groups of four daughter corpuscles (fig. 21).

In respect of this peculiar quadri-sectional division, the chlorophyll-corpuscle of the vegetating Archerina very closely resembles that of Hydra viridis, as may be seen by a

comparison of fig. 25 of the present Plate VII with fig. 17 *a* of Pl. XX, Vol. XXII (1882) of this Journal.

The chlorophyll-corpuscle of Archerina has accordingly an interesting relation to the question which has been raised by Brandt, as to the parasitic nature of the chlorophyll-corpuscles of Hydra viridis. If the theory is entertained that the latter are independent green algæ which inhabit the endoderm cells of Hydra as parasites, then it would seem necessary to take a similar view with regard to the chlorophyll-corpuscle of Archerina. The Archerina would itself be a very simple non-nucleate Gymnomyxon similar to Vampyrella, which would be supposed to be always inhabited and dominated in its movements of growth and division by the green algal parasite. With regard to such a conception, it may be justly observed that on equally valid grounds the nuclei of other Protozoa—and, indeed, of all animal and vegetable cells—might be regarded as colourless parasites inhabiting non-nucleated corpuscles of protoplasm. On the other hand, it would be urged that no independent organisms resembling the nuclei of cells are known, and that in the absence of any direct evidence of their intrusion from external sources into the protoplasm of cells, as well as in view of the phenomena of their division and their relation to the protoplasm, it is a gratuitous assumption that they have a history differing essentially from that of other products of the modification of cell-substance. In the same way we urge, in reference to the tetra-schistic chlorophyll-corpuscles of both Archerina and Hydra viridis, that they do not resemble any known unicellular green alga, either in structure or in mode of growth, and that there is no reason for attributing to them a fanciful origin and history differing essentially from that of other coloured corpuscles and such products of the modification of cell-substance.

2. **Encysted Form** (figs. 1 to 6).—In the earlier gatherings sent to me by Mr. Bolton, which contained only a few of the vegetating growths of Archerina, and these only in the condition of small colonies, with four or eight chlorophyll-corpuscles, I found many specimens of large encysted Archerinæ. These

also were again obtained from a tube which contained at first abundant colonies, such as that drawn in Pl. VII, fig. 24. After a week's interval the colonies were found to have broken up into single individuals (that is, individuals containing each but one chlorophyll-corpuscle), and a week later many were found to have increased greatly in size, and to have become enclosed in a cyst. The cyst appears to consist of a resisting membrane, which is produced on the surface of the protoplasm, and actually extends for some distance along the filamentous pseudopodia. As the deposit increases in amount the pseudopodia are withdrawn, and finally there results a spherical cyst provided with numerous truncated processes on its surface, resembling the short spines of a horse-chestnut fruit. In such cysts the protoplasm and its chlorophyll body may be observed in various conditions. I have most frequently found the protoplasm shrunken, so as to be completely detached internally from the cyst-membrane (figs. 2 and 3). At the same time, in such specimens there was no appearance of any differentiated chlorophyll-corpuscle, but the whole of the protoplasm was uniformly coloured green. Occasionally I have seen the whole contained mass in a state of granular disintegration (fig. 1). On the other hand, the cysts sometimes show (figs. 4, 6) a disposition of the protoplasm with vacuole and two chlorophyll-coloured masses not dissimilar to that of the un-encysted Actinophryd form (fig. 7).

I am not able to state what is the exact position of the encysted condition in the life-history of Archerina.

I do not know whether the Actinophryd-forms, such as figs. 7 and 12, are just about to be encysted, or whether they have just escaped from the encysted condition. The latter seems to me to be the more probable.

3. Vegetative Condition—Tetraschistic Colonies (figs. 20, 21, 22.)—Alongside of the Actinophryd-forms of Archerina occur very numerous specimens in which the protoplasm is no longer so definitely disposed in the form of a central sphere and clean-cut radiating filaments, but is irregular in shape with occasional lobose projections, whilst groups of radiating filaments are

given off here and there from the mass. In these specimens the most striking feature is the entirely altered appearance of the chlorophyll-corpuscles. They may be present to the number of four, arranged as in fig. 15, and clearly resulting from the tetraschistic division of one parent chlorophyll-corpuscle. Or they may have proceeded further in the process of fission, each one of four having itself divided into four, giving thus a group of sixteen, such as is shown in fig. 21. These may retain a very definite and symmetrical arrangement, or the colony may have become broken and distorted so as to give such irregular grouping as is shown in fig. 20. That there is at one stage or other a possible division into two only on the part of the chlorophyll-corpuscle of Archerina, is shown by the existence of such a group as that drawn in fig. 22, where we have a colony consisting of four groups of eight corpuscles. A curiously irregular fission is shown in fig. 23.

The two corpuscles with abundant protoplasm shown in fig. 19, are very possibly only a detached "half" of a tetraschistic group.

It is to be noted with regard to the form of the chlorophyll-corpuscles in these groups, that they contrast with the oval and irregularly flattened out green bodies of the Actinophryd-phase. They are nearly always spherical, rarely ovoid. Occasionally, as in fig. 20, each contains a refringent granule, but they are usually homogeneous in appearance. The chlorophyll is confined to a uniform peripheral layer or crust of the corpuscle.

The spherical form of the corpuscles is apparently connected with their active state of growth and division, the breaking up of one parent corpuscle into four daughter corpuscles proceeding rapidly, and not being delayed in the incomplete state of fission seen in the Actinophryd-phase. The protoplasm is more abundant relatively in these groups than in the Actinophryd-phase, and is often observed in the act of ingesting solid food particles such as Bacteria (seen in fig. 20, i, and fig. 24, i). That the multiplication of the corpuscles and the associated growth of the protoplasm is very active, seems to be proved by the occurrence of such extensive growths of the organism as

that drawn in fig. 24. This is by no means the largest colony which I observed, and in this specimen the chlorophyll-corpuscles were all in an active state of tetraschistic division. I decolourized this colony by the introduction of alcohol between the glass slide and cover, whilst under observation, and, subsequently stained the organism by picro-carmine introduced in the same way. All the larger chlorophyll-corpuscles then exhibited the structure shown in fig. 25. Whilst the smaller corpuscles (which presumably had only recently been formed by tetraschistic division) exhibited the structure shown in fig. 26. The shaded parts in these two figures correspond to a decided but not very strong staining effected by the picro-carmine.

The size of the chlorophyll-corpuscles in these vegetative groups varies. They are never so large as in the Actinophryd phase, but may attain a diameter of $\frac{1}{4000}$th of an inch, and may be, in such exceedingly active specimens as fig. 24, as low as $\frac{1}{16000}$th of an inch.

It appears that there is no constant size which the chlorophyll-corpuscle must attain before division, but that this varies in different specimens according to the individual activity of the vegetative process in each group. Apparently, where the protoplasm is abundant and is taking much nourishment, the chlorophyll-corpuscles multiply rapidly and enter upon the fission-process at an earlier period of growth—that is to say, when they have attained a less diameter—than is the case where the protoplasm is less abundant. It would almost seem as though, receding from the Actinophryd-phase, the chlorophyll-corpuscles divide successively at earlier and earlier stages of their growth, until a maximum of associated corpuscles and a minimum of their individual size is attained. It seems not improbable that this excessive growth and subdivision of the chlorophyll-corpuscles is favoured by abundant nutrition. A time arrives when the conditions for nutrition are less favourable. The individual chlorophyll-corpuscles then grow instead of dividing and each becomes detached, together with some of the protoplasm, from association with its neighbours. Such a

growth as fig. 24 would break up into several hundred individuals. Each of these then would slowly attain to the size and form of the Actinophryd-phase (fig. 7), the chlorophyll-corpuscle partially dividing and spreading itself out as seen in figs. 7, 8, 9, 10, 11.

Probably such individuals now pass on—if conditions adverse to nutrition are continued—into the encysted condition, from which they will emerge on the return of conditions favorable to nutrition.

This life-history is hypothetical. It is, however, favoured by the fact that specimens of vegetative growths such as fig. 24, when kept in the moist-chamber for two weeks, first of all broke down into individual units consisting of a single chlorophyll-corpuscle and some protoplasm, and that later many were observed of a large size in the Actinophryd-phase, whilst later still on the same slide numerous encysted individuals were found which were not previously detected. A similar observation was made with regard to the contents of a glass tube kept on the table of my laboratory screened from the direct sunlight.

4. Skeleton-colonies.—A very curious characteristic of the colonies of Archerina is represented in fig. 18. In exploring a slide containing specimens in the early tetraschistic phase (such as figs. 20 and 22), one comes across groups of ghost-like outlines corresponding to chlorophyll-corpuscles, and their radiant filamentous pseudopodia, entirely devoid of any substance. They are merely outlines, as though sketched with a pencil, very delicate and inconspicuous. Here and there in such a ghost-like group one finds a solid chlorophyll-corpuscle and attendant protoplasm.

These strange outline "simulacra" of Archerina-colonies are undoubtedly skeletal products of the solid protoplasm, which after producing them has withdrawn from them and moved into another position. They appear to indicate that the protoplasm is at this phase of the life-history of Archerina capable of producing a pellicle on its surface, comparable to the cyst which is produced when encystation takes place; but instead

of being retained as a covering for a definite period, the
secreted material is soon abandoned by the organism, and thus
these ghostly sketches of the Archerina are left empty and
useless. They may be compared to the numerous cellulose -
chambers secreted and rapidly abandoned by the protoplasm of
Archer's Chlamydomyxa. They are remarkable inasmuch as
they show that the whole surface of the protoplasm of Arche-
rina can secrete a skeletal product. Not only the delicate
layer of protoplasm which invests each chlorophyll-corpuscle,
but also the filamentous pseudopodia as far as their delicate
extremities secrete this skeletal investment. The secretion
of a skeletal investment by filamentous pseudopodia is unusual.
It is known to occur in such oceanic Thalamophora as Glo-
bigerina, where the investment is calcareous, and in some
Radiolaria (Tripylæa) where it is siliceous (hollow spicules).

A membranous investment secreted by pseudopodia is, I
believe, hitherto unobserved. The Heliozoa are known to
obtain a certain stiffness and permanence for their filamentous
pseudopodia by the secretion of an axial horny filament.
Here we seem to have evidence of a capacity for strengthening
and stiffening the radiant pseudopodia, by the development of
an external skeletal tube of a similarly horny (membranous)
nature.

As to how the living matter recedes from the investment
which it has formed so as to leave these empty cases, I have no
suggestion to offer. And I am not able to assert, although it
seems to be unlikely, that the removal of the living matter
from within these ghostly skeletons may not be due to the
death and decomposition of the living matter. In any case,
these skeletal residues of Archerina-colonies are amongst the
most interesting and characteristic of the features presented by
this organism.

5. **Physiological Observations.**—The protoplasm of Archerina
in all the phases here recorded was extremely sluggish. I did
not detect any streaming movement in it in any case, nor any
change of form which could be followed with the eye. In the
Actinophryd-phase I failed to observe any evidence of the

ingestion of food particles, but in the later vegetative growth I often saw Bacteria and Bacilli in course of ingestion (figs. 20, 24, *i*).

No contractile vacuole was observed in any phase.

In the Actinophryd-phase only was a vacuole (and that a non-contractile one) observed. The protoplasm in the Actinophryd-phase is free from granules, homogeneous and refringent. In the vegetative stage it has a finely-flaky appearance.

The pseudopodia were not altered in form by the action of dilute acids or of alcohol.

Numerous observations were made as to the presence of amyloid substance in connection with the chlorophyll-corpuscles. In small colonies I usually failed to obtain any violet coloration after removal of the chlorophyll by alcohol and subsequent addition of iodine solution. But in larger colonies I obtained decided violet staining of the protoplasm immediately surrounding the chlorophyll-corpuscles, for instance, in such colonies as that represented in fig. 24.

6. **Affinities of Archerina.**—Archerina is clearly one of the non-nucleate Gymnomyxa (Homogenea or Monera), and is, in so far as regards the various forms which its protoplasm may assume, not far removed from Cienkowski's Vampyrella. It is, however, definitely characterised and distinguished by its nucleus-like chlorophyll-corpuscle. No other Protozoon is known the form of which is thus dominated by a chlorophyll-corpuscle, nor is there any form with a chlorophyll-bearing nucleus which might be compared with it. In regard to nutrition it clearly gives evidence of both plant-like assimilation of carbon through the agency of its chlorophyll-corpuscles and of the usual ingestive voracity of the naked Protozoa.

In respect of its abundant colony-formation, Archerina reminds one of Microgromia socialis; but it differs widely from that organism in having a chlorophyll-corpuscle in place of a nucleus, and in forming a complete membranous envelope extending over the pseudopodia instead of (as in Microgromia) a sac-like case with a mouth or orifice for exit.

I shall not be surprised if some naturalists maintain that

Archerina is a duplex organism consisting of a Moner-like animal Protozoon, and a simple green alga, living together in constant association, or "Symbiosis." But in my judgment there is no direct evidence, nor are there any grounds of analogy, for entertaining such a view as to the nature of this organism.

EXPLANATION OF PLATE VII,

Illustrating Prof. Ray Lankester's memoir on "Archerina Boltoni," nov. gen. et sp.

Fig. 1.—Cyst of Archerina Boltoni, in optical section. *a.* Cyst wall. *b.* Granular contents, coloured green by chlorophyll. Nat. size = $\frac{1}{1800}$th of an inch in diameter.

Fig. 2.—Another cyst, similarly viewed. *a.* Cyst wall. *b.* Protoplasm withdrawn from the cyst wall, and uniformly coloured by chlorophyll. *c.* Long tubular processes of the cyst.

Fig. 3.—Surface view of a similar cyst.

Fig. 4.—Optical section of another encysted Archerina, in which the colourless protoplasm *d* is distinguishable from the two chlorophyll bodies *b b.* Other letters as in Fig. 2.

Fig. 5.—Optical section of another cyst, showing uniformly short processes of the cyst wall, and green-coloured contents entirely free from the cyst wall. Letters as in Fig. 2.

Fig. 6.—A deeper focussing of the specimen drawn in Fig. 4, showing the constricted vacuole *e* within the protoplasm. Other letters as before.

Fig. 7.—Actinophryd-phase of Archerina Boltoni. Diameter of the sphere $\frac{1}{2000}$th of an inch. *b b.* Chlorophyll bodies. *e.* Vacuole.

Figs. 8—12.—Central spheres of different specimens similar to Fig. 7, the radiating pseudopodia being omitted. They show various dispositions of the chlorophyll and of the vacuoles.

Fig. 13.—Similar specimen to Fig. 7, showing two large chlorophyll bodies and a small vacuole.

Fig. 14.—Similar specimen in a stage of tetraschistic division. The radiant protoplasm is omitted, and the pyramid of four incipient segmentation spheres is seen from one face.

Fig. 15.—Four segmentation spheres resulting from a complete tetraschistic fission of the chlorophyll-coloured body of an Actinophryd-phase of Archerina.

The uncoloured protoplasm is relatively small in amount and is not seen, owing probably to its forming in part a thin coating to the chlorophyll-corpuscles, and being in part accumulated between and beneath those bodies.

FIG. 16.—Archerina of same size as Fig. 7, but with irregularly-shaped chlorophyll-corpuscle, and with protoplasm gathered partly into an amœboid lobe and partly into one long filament.

FIG. 17.—A specimen of same size, showing few but large filamentous pseudopodia, and with a chlorophyll-corpuscle sharply cleft into two.

FIG. 18.—Skeletal or "ghost" colony of Archerina. The spheres and filaments marked h are merely empty cases of great tenuity. Four chlorophyll-corpuscles are present and lobose protoplasm g. Diameter of each sphere = $\frac{1}{4000}$th of an inch.

FIG. 19.—Colony consisting of two spherical chlorophyll-corpuscles surrounded by radiant and, g, lobose protoplasm.

FIG. 20.—Irregularly-grouped chlorophyll-corpuscles and protoplasm, resulting from tetraschistic division of an originally single chlorophyll-corpuscle, such as that seen in Fig. 12 or 16. The abundant amœboid protoplasm g is actively ingesting a bacillus-filament i.

FIG. 21.—Tetraschistic colony of Archerina consisting of four groups of four chlorophyll-corpuscles, each invested with radiating filamentous protoplasm. The regular symmetrical grouping of the products of division is striking, though not unusual in this organism.

FIG. 22.—A similarly symmetrical colony, consisting of four groups of eight chlorophyll-corpuscles.

FIG 23.—Small colony, in which the division of the chlorophyll-coloured spheres has not proceeded symmetrically, the product being one large and three small spheres. This is a very exceptional condition.

FIG. 24.—Large vegetative growth of Archerina, consisting of some hundreds of chlorophyll-corpuscles and amœboid protoplasm, g, giving off filamentous radiant pseudopodia at many points. Bacilli, ii, are being ingested by a portion of the protoplasm. The chlorophyll-corpuscles are arranged more or less obviously in groups of eight or sixteen, the arrangement resulting from their method of multiplication. They are of two sizes, the larger measuring $\frac{1}{8000}$th of an inch in diameter, the smaller ones, k, less. The larger are seen in many places to be in course of breaking up to form the smaller by tetraschistic division.

FIG. 25.—One of the larger chlorophyll-corpuscles of the specimen drawn in Fig. 24, after removal of the chlorophyll by alcohol and subsequent staining with picro-carmine. The fission lines of tetraschistic division are seen.

FIG. 26.—One of the smaller chlorophyll-corpuscles of the same specimen similarly treated.

On the Apex of the Root in Osmunda and Todea.

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With Plates VIII and IX.

Introduction.

The years 1845 and 1877 will always be memorable in the history of the study of apical meristems. In the former year Naegeli published in his work, 'Die neuern Algensysteme,' his investigations on the mode of growth of certain Algæ, notably of Dictyota dichotoma, and introduced the term apical cell (Scheitelzelle) to distinguish that cell from which segments are cut off in regular order and succession, these segments giving rise, by further growth and division, to all the mature tissues of the organ. In 1877 Sachs produced the first of that series of memoirs[1] which drew together and systematised the immense volume of independent results obtained by various investigators of meristems during the previous thirty years. It is true that Hofmeister ('Lehre von der Pflanzenzelle,' 1867, p. 125, &c.) had attempted to draw from a comparison of various meristematic tissues, some general conclusions as to the relations of cell divisions to growth, but these

[1] 1. "Ueber die Anordnung der Zellen in jüngsten Pflanzentheilen," 'Verh. der Phys. Med. Gesellsch. in Würzburg,' Bd. xi, 1877.

2. 'Arbeiten des Bot. Inst. in Würzburg,' Bd. ii, Heft i.

3. 'Arbeiten des Bot. Inst. in Würzburg,' Bd. ii, Heft ii.

4. 'Vorlesungen über Pflanzenphysiologie,' pp. 523—557.

were subjected to severe criticisms by Sachs at the opening of
the second memoir of the above-mentioned series (l. c., p. 48).
Thus, practically, the ground was clear for the latter author.
How well he drew together and systematised the whole sub-
ject of the structure of meristems will be already familiar to
most botanists. It may be safely stated that no work has given
a greater stimulus to the study of meristematic tissues than
this of Professor Sachs.

One of the most important points brought forward by Sachs
is that the arrangement of the walls in such organs as grow
with a single apical cell may be shown to fall under a similar
system of construction to that of similar organs in which no
apical cell can be distinguished. He has formulated this in
the proposition that the apical cell "is merely a gap in the
system of construction of the punctum vegetationis, that
is, the apical cell is that point in the embryonic tissue in which
as yet no anti- or periclinal walls, nor any radial longitudinal
walls have been formed" ('Vorlesungen,' p. 555). This view
of the matter affords a bridge connecting those types which
grow with a single apical cell with those having a small-celled
primary meristem, and as a necessary result draws closer atten-
tion to those which may be regarded as transitional types
between the structure which is, roughly speaking, characteristic
of the lower forms, where an apical cell is present, and that
more typical of the higher plants, viz. with a small-celled me-
ristem. Such intermediate types had already been described
in various examples, both of roots and stems, in which the
whole tissue of the organ, instead of being referable in its
origin to a single apical cell, is derived from two or more
initial cells. As examples may be cited the roots of the
Marattiaceæ, first investigated by Russow ('Vergl. Unters.,'
pp. 107—109), but subsequently, and apparently with more
exactitude, by Schwendener ('Sitz. d. k. Preuss. Akad. d.
Wiss.,' 1882, p. 183). According to the latter author, there
are in this case four oblong initial cells in juxtaposition ; with
the exception of the sides in contact with the other initial
cells, segments are cut off from all the sides of these cells ;

those cut off below (i. e. from the end next the body of the root) take part in the formation of the body of the root, those above act as calyptrogen.

A group of initial cells, having apparently very similar characters to the above, but without the formation of a calyptrogen, has recently been described by Bruchmann (ref., 'Bot. Centrbl.,' 1884, No. 46), in the stem of Selaginella spinulosa, though it had already been noted by Sadebeck ('Schenk's. Handbuch.,' Bd. i, p. 244). In Selaginella Wallichii Strasburger found that two wedge-shaped initial cells occupy the summit of the stem.[1] Finally, Strasburger, in his recently published 'Botanische Practicum,' gives a drawing (l. c., fig. 93) of the apex of the stem of Lycopodium selago, in which three cells (marked *i*) are distinguished as initial cells. Two of these are again represented as seen in longitudinal section in his fig. 94. A useful summary of different varieties of such meristems is to be found in Haberlandt's 'Physiologische Pflanzenanatomie,' pp. 44—52. In a note on p. 59 of this work the question of the mode of transition from growth with one apical cell to that with two or more initial cells is discussed, but owing to the want of necessary observations on the subject it is only treated theoretically. As yet we have no direct evidence as to the manner of transition, though, as Haberlandt points out (p. 60), the transition must have taken place. It is the object of this article to add to the information at present at our disposal.

In a memoir 'On the Comparative Morphology of the Leaf in the Vascular Cryptogams and Gymnosperms,' communicated to the Royal Society, I have pointed out that in various characters of the leaf the Osmundaceæ occupy a position intermediate between the leptosporangiate Ferns and the Marattiaceæ. The idea suggested itself that the Osmun-

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[1] When it is remembered that Treub found a single wedge-shaped apical cell of variable form in the stem of Selaginella Martensii, it will be clear that in this genus variations of structure of the apical meristem of the stem are to be found which are very similar to those described below in the Osmundaceæ.

daceæ might also be a transitional type in respect of the
structure of the meristem in the root. The result justified my
expectations, and the observations to be detailed below will
afford material help towards the solution of the problem as to
the mode of transition from growth with one apical cell to that
with a group of two or more initial cells.

Osmunda regalis.—Transverse sections.

In describing the manner in which the arrangement of the
primary meristem of the root of Osmunda regalis differs
from that generally accepted as characteristic of the roots of
Ferns,[1] the results obtained from transverse sections will be
detailed first. As the irregularities are in some cases very
great, and as there is not uniformity of structure in the meri-
stem of different roots of this species, even when taken from
the same plant, it is obvious that, in order to attain a clear
idea of the irregularities, the study of transverse sections is
more likely to lead to safe conclusions than that of longitudinal
sections. Anyone who has made preparations from the roots
of those Ferns which have an almost diagrammatic regularity of
arrangement of the meristem, will, I think, allow that the study
of transverse sections of these is more easy and secure than
that of longitudinal sections. But if there be no definite regu-
larity of arrangement of the meristem the difficulties presented
by the study of longitudinal sections are very greatly increased,
since it is in this case so much less easy to determine whether
any given section be accurately longitudinal, and almost im-
possible to be sure whether it be accurately median. In ob-

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[1] Naegeli and Leitgeb, 'Beiträge z. wiss. Bot.," Leipzig, 1868. It may
here be stated that I have made sections from the apices of roots of Cyathea
insignio, Eat., Gleichenia circinata, Sw., Gleichenia flabellata,
R. Br., and Aneimia phyllitidis, Sw., and have found in all of these
that the structure of the meristem corresponds in its chief points to that
described by Naegeli and Cramer for the Polypodiaceæ. It is clear that the
prevalence of this type of meristem among the leptosporangiate Ferns makes
the abnormality of structure in the Osmundaceæ still more interesting than it
would otherwise have been.

serving transverse sections of roots of irregular meristematic structure care must be taken to distinguish sections passing through the root cap, immediately above the actual initial cells, from those passing through the initial cells themselves. It is well known that investigators have at times fallen into error on this point. In all cases I have mounted all the transverse sections cut from one root on the slide together, and have not drawn conclusions from any one section till it has been compared with the other sections of the series, and its actual position in the original root been thereby defined. Though the grosser errors have, I think, been excluded by due care on this and other points, still the difficulties arising from want of uniformity of meristemic structure make absolute certainty in the interpretation of sections, and especially of longitudinal sections, almost impossible. Errors of detail, both of observation and interpretation, may have crept in, but still the description which follows will amply show that the structure of the meristem of the roots in Osmunda and Todea may differ essentially from the type described by Naegeli and Leitgeb (l. c.).

Among the many roots of Osmunda regalis which have been investigated, some few show a similarity in the arrangement of the meristem to the type well known for Equisetum and the Polypodiaceæ. These will be first described. The most regular which has been observed is that represented in fig. 1 ; unfortunately, this section was not cut exactly in a transverse plane, and hence, as regards their form, the segmental cells are not so regular as they would otherwise have appeared ; still it is clear that there is in this case a three-sided apical cell, from the three sides of which segments I—IV have been cut off in regular order. It is worthy of note that this was one of the thinnest of all the roots from which sections were cut ; it measured $\frac{22}{1000}$ths of an inch in transverse diameter at the level of the section : the question as to the relation of bulk of the root to the arrangement of the apical meristem will be discussed more at length below.

Three examples of one four-sided apical cell have been

observed, two of which are represented in figs. 2 and 3.   In
the former the square group of cells figured ocupied a central
position, while the succession of segments was regular.   In
fig. 3 the succession of segments is as regular as in the former
case, till the segment IV is reached ; then it appeared that
a second segment (V) had been cut off from the same side
of the apical cell as segment IV.   The appearance of the
walls as shown in the figure might suggest a possible interpre-
tation of the meristem as being derived from four initial cells,
as described by Schwendener in the roots of the Marattiaceæ;
a careful examination of the section precludes this idea.   It is
further to be observed that the section represented in fig. 3,
passed through the apical cell with its segments, while it also
included a segment (already divided into four cells by walls
disposed crosswise, and here shown by dotted lines) which goes
to form part of the root-cap : this may be observed by focussing
deeply into the section.   Such sections, when used with proper
precautions, are of great value, as preventing the possibility of
mistaking cells below the apical cell, or group of initial cells,
for the apical or initial cells themselves.

Occasionally intermediate examples are found between the
type with a single well-marked apical cell, and more complex
arrangements.   In the sections represented in figs. 4 and 5,
neither the position of that cell which is probably the single
apical cell, nor the arrangement of the tissue round it, give
evidence of the growth having been strictly according to rule.
Thus in the case represented in fig. 4, the three-sided cell
marked (x) does not hold a central position in the section, and
it is of relatively small size : here the appearance of the tissue,
and the arrangement of the surrounding cells, suggest rather
that the four-sided cell marked (x) has performed the function
of an initial cell.   Another example of irregularity is shown
in fig. 5, B.   The cells marked (x) hold a central position, and
appear to be sister cells ; the surrounding tissue does not point
to any definite regularity of succession of segments cut off
from them.

Of all the modes of arrangement of the cells of the apical

meristem found in Osmunda regalis, that which is perhaps
the most interesting from a theoretical point of view, is that
represented with varying clearness in the figs. 6, 7, and 8.
In each of these figures it may be seen that the whole tissue
is referable in its origin to three initial cells, which are marked
(x), while the three walls separating these cells from one
another meet at a central point. These three walls are drawn
in heavier lines, and may be traced for some distance from the
central point; they may be called the principal walls (P in
the figures, Cf. Hauptwände, Naegeli and Leitgeb). In each
case the portion of tissue derived from one of these initial cells
is enclosed between two of these principal walls, and thus the
whole meristem may be regarded as consisting of three wedge-
shaped masses. Taking now those masses singly into consi-
deration, it will be clearly seen that in fig. 6 each is divided
into two unequal parts by walls marked (s), which do not
proceed to the centre, but on passing towards it curve
gradually out of the radial plane, and insert themselves at
right angles on the principal walls : these may be called the
sextant-walls (Cf. Sextandenwände, Naegeli and Leitgeb.)
A similar arrangement, but less clearly marked, may be seen
in such examples as those represented in figs. 7 and 8. In each
case the error of regarding sections below the real initial cell
or cells as including the initial cells, has been carefully
avoided; that the fig. 8 actually represents the apical group,
and not apart of the tissue below it, is proved by the fact that
the section includes division walls characteristic of the root
cap, one of which is represented by the dotted line; this comes
into view only on focussing deeply into the section : the cell
walls drawn in this figure as continuous lines are those seen on
observing the section from the side more remote from the
root cap.

For comparison with this arrangement of the initial cells and
their derivatives I have quoted, as figs. 9 and 10, two of the
drawings of Naegeli and Leitgeb, which appeared in their well-
known work, ' Entstehung und Wachsthum der Wurzeln,' as
Taf. xii, fig. 8, and Taf. xiv, fig. 5. These represent optical

transverse sections of the roots, in the former case of Equisetum hiemale, in the latter of Pteris hastata, at points immediately below the apical cell. The correspondence of arrangement of the cells in these sections with that of the apical meristem of Osmunda, as seen in figs. 6—8, is undeniable, though certain irregularities, which are less common in Equisetum and Pteris, are frequent in Osmunda. Thus it will be observed that the sextant walls in the former usually curve all in the same direction, and are homodromous, according to the terminology of Naegeli and Leitgeb (l. c., p. 105) ; they accordingly insert themselves successively on the three principal walls. This is not the rule in Osmunda (figs. 6 and 7), but a similar irregularity is to be observed in the fig. 9, of Equisetum, in which two of the sextant walls are inserted on opposite sides of one of the principal walls. This heterodromous arrangement (Naegeli and Leitgeb, l. c., p. 105) of the sextant walls, which appears to be the exception in Equisetum, is not unfrequent in Osmunda. The further consideration of this interesting point must be deferred for the present.

Before leaving the study of transverse sections it may be observed that they show that the divisions of the segments which go to form the root-cap appear to be so arranged that, whatever the form or number of the initial cells, each successive addition to the root-cap consists of a group of four cells. This is illustrated in figs. 3 and 7, in which the group of four cells may be recognised, though derived from initial cells of very different types. A similar result was obtained by Naegeli and Leitgeb, in their observations on Equisetum and the Polypodiaceæ.

Lastly, I may state that no clear case of four initial cells, such as have been described by Schwendener for the Marattiaceæ (l. c.), has been observed by me in any of my preparations from the root of Osmunda regalis.

Longitudinal Sections.

In organs having a single apical cell, from which segments of definite form are cut off in regular succession, the position and appearance of the apical cell itself, and of its segments when seen in longitudinal section, may be used as a test whether the longitudinal sections of the apex be accurately median or not; but where there is no rigid regularity of form of the apical cell, or where there may be more than one initial cell present, the difficulty of judging whether a section be median or not is greatly increased. I have shown by means of transverse sections that the latter is the case in Osmunda; that where a single apical cell is present it is not always of uniform shape; and, further, that the number of initial cells may be as high as three. Moreover, it is not asserted that the varieties which may occur are by any means exhausted by the foregoing description. This being the case it is necessary to approach the study of longitudinal sections with great caution, and to subject the results to strict criticism. It is impossible to expect that there will be uniformity in the arrangement of the cells, as seen in longitudinal section, and the observations about to be detailed show that uniformity does not exist. As in the case of transverse sections, so also in observing longitudinal sections all the approximately median sections cut from one root were mounted and examined, usually on both sides, before conclusions were drawn. But beyond the mere difficulty of ascertaining the structure of the varieties of this irregular meristem, there remains that of suggesting to which of the types of structure seen in transverse section any given one seen in longitudinal section most nearly corresponds. I do not profess to any near degree of certainty on this point, and where an opinion is put forward it must be understood that it is advanced only in a tentative manner.

In the majority of cases cells of a pyramidal form have been found occupying an approximately central position in median sections; but in no single instance has an apical cell been observed having that regularity of form, and of arrangement

of its segments, together with the nearly central position which are so characteristic of the roots described by Naegeli and Leitgeb. Taking as the first examples (figs. 11, 12) two of those which approach most nearly to that type, the pyramidal apical cells (x) can be readily recognised in each case; but a comparison with the figures of Naegeli and Leitgeb shows certain points of difference. In the first place there is a difference in the form of the cell itself; it is in Osmunda proportionately narrower and deeper, that is, more elongated in a longitudinal direction, and consequently the principal walls by which it is bounded laterally are less inclined to the longitudinal axis. Secondly, the arrangement of the cells surrounding the pyramidal cell does not show any definite regularity, and it is thus difficult to ascertain their genetic connections. This irregularity is found not only in the tissue adjoining the sides of the pyramidal cell, but also in those which have been derived from its base, and will go to form tissues of the root-cap. Comparing these figures with Naegeli's, though the similarity is obvious in its main points, the regularity of detail so characteristic of the plants he described is absent even in these, which are the most regular examples of the apex of the root of Osmunda which I have observed.

A further point to be noted in figs. 11 and 12, but which is much more prominent in figs. 13 and 14, is that in point of size the wedge-shaped cell is smaller in comparison with the adjoining cells, than is the case in other Ferns; whereas in Naegeli's figures the area of the apical cell exceeds that of any of the surrounding cells, in Osmunda it is as a rule smaller than they. It may be observed not unfrequently that one cell of a group, obviously of sister cells, takes the lead in point of size, the form of such a cell being usually a truncated pyramid, as shown in fig. 15 (x). I think it probable that in such cells we may trace an ascendancy in more than mere size; in fact they may have, in part at least, the function of initial cells. This would appear to be more clearly the case in fig. 13. In the apex represented in fig. 14, the pyramidal cell is clearly seen; here, though there is no marked ascendancy of

the neighbouring cells in point of size, still if the genetically connected groups of cells, enclosed between the darker marked anticlinal walls, be taken into consideration as products of successive segments, it is clear that the function of the apical cell is comparatively in abeyance, while the formation of new tissue is conducted with unusual activity by the cells derived from recent segments. Pyramidal cells, other than the apical cell, which appear at points removed from the organic centre (figs. 13 and 14, marked *o*), do not seem to have any functional importance different from that of the cells immediately surrounding them.

From the example shown in fig. 13 to that in fig. 16 the transition is easy. In the former case there are two rather irregular pyramidal cells of unequal size (x, x.), in the latter there are two such cells, of regular form and equal size, which overlap one another as seen in this section; there is in the latter case a greater regularity in the subdivision of the segments than is usually to be found in the roots of Osmunda.

Lastly, the less common, but very interesting arrangement shown in figs. 17 and 18 must be mentioned. Here no pyramidal cell is to be found; the median longitudinal section shows two cells (x, x.) of truncated pyramidal form, from which segments are cut off, (1) from the base, to form tissues of the root-cap; (2) from the sides, and, (3) as shown in fig. 17, from the truncated apex also. In this case the correspondence with Schwendener's description of the apical group in the root of the Marattiaceæ is very apparent.

It has been demonstrated repeatedly by various authors that in the cases of Equisetum and many Ferns there is a certain order of succession and regularity of position of those walls by which the segments are divided up into smaller cells. A comparison of the drawings above quoted, with those of Naegeli and Leitgeb, will suffice to show that no such regularity is found in Osmunda, even where a pyramidal cell is present; the continuity of the procambial tissue may be traced up to a point close to the apical cell or group of cells, those cells which are about to form tracheides being easily recognised at

very early stages (figs. 11, 16, 17, *tr.*) ; but the outer limit of
the procambial cylinder does not appear to correspond to any
definitely recurring wall in the young segments, as is the case
in the roots investigated by Naegeli. The same may be said
with respect to the limits of the cortex and epidermis ; the
latter tissue, which can readily be recognised in the older part
of the root, and can be traced as entering below the layers of
the root-cap, loses its identity at a considerable distance from
the actual organic axis of the meristem. This fact is illus-
trated in the figs. 11 and 18 ; in neither of these examples can
a clear distinction be drawn between those cells which will
develop as tissue of the root-cap and those which will form
epidermis, or cortex.

It remains to suggest which of the several types of arrange-
ment seen in longitudinal section correspond to the several
types above described as being seen in transverse sections, but
it must be understood that what follows is only put forward in
a tentative manner. The observation of thick transverse sec-
tions appears to show that where there is a three-sided apical
cell, it is of pyramidal form; whether the same is the case with
four-sided apical cells is uncertain. On the other hand, where
the number of initial cells is three (or possibly in some cases
four), transverse sections lead to the conclusion that they are of
the truncated pyramidal form. Thus it is probable that where
a pyramidal cell is seen in longitudinal section, it is a cell of a
three-sided or four-sided pyramidal form. Probably, also, the
pyramidal cells marked (*o*) in figs. 13 and 14 correspond to
such cells as those marked also (*o*) in the transverse sections
fig. 5, B, and fig. 4. Further, on the grounds above mentioned,
it seems probable that arrangements such as those in figs. 17
and 18 correspond to such meristems as those represented in
transverse section in figs. 4 and 7.

A question of considerable importance is whether in the
individual root a transition may occur at any time from one
of these types of meristematic structure to another. That a
number of roots of a given species or individual differ in
meristematic structure from one another is no argument

against an approximately uniform structure of the individual
root throughout its development.   It is impossible to decide
the question with absolute certainty, but some collateral
evidence may be gathered from the study of the origin and
early development of lateral roots; if there be uniformity in
the first divisions of the rhizogenic cells, while there is irre-
gularity of structure in the older roots, a transition of struc-
ture must necessarily have occurred.   With the object of
ascertaining this point, the origin of lateral roots has been
investigated, and certain well-marked examples of the arrange-
ment of the early divisions of the rhizogenic cell are shown in
figs. 19 to 22.   As is the case in other Ferns, the lateral root
of Osmunda originates from a single initial cell, which
belongs to the endodermis, and is situated opposite one of the
groups of Xylem.   Fig. 19 shows how this rhizogenic cell has
divided by walls inclined to one another, so that from the very
first there is a pyramidal apical cell in this young root.   Fig.
20 shows a similar pyramidal cell, but in this case the young
root was further advanced, and its apex had penetrated to the
outer limit of the cortex of the main root.   On the other hand,
in the young lateral roots represented in figs. 21 and 22 there
are no pyramidal cells to be seen, the young roots in these
cases appear to show an arrangement of initial cells (x, x)
similar to that in figs. 17 and 18.   Thus, in the very first
stages of development differences of meristematic arrangement
may appear, which are quite as great as those between the
most extreme types of structure described above for the apex of
the more mature root. This observation, it is true, affords little
more than negative evidence ; we may conclude from it that
since as great varieties of meristematic structure are to be
found in the roots at their first stages of development as those
seen in more mature roots, it is therefore unnecessary to
assume a transition of meristematic structure from one type
to another in the individual root, in order to explain the
differences of structure in the mature root. We have, how-
ever, no evidence which precludes the idea that such a transi-
tion may actually take place ; all the evidence being taken

into consideration, it seems probable, though not proved, that there is variation in meristematic structure, within certain limits, in the individual root during its development just as in Selaginella Martensii, as demonstrated by Treub, the form of the apical cell is liable in the individual stem to certain variations of form.

The study of the anomalous structure of the meristem of the root of Osmunda affords us information as to another question, which has an important bearing upon views as to the connection between external form and internal cellular structure. The question is this—whether any constant relation is to be found between the bulk of the individual root and the type of structure of its meristem? In order to answer this question for the roots of Osmunda regalis the sections from which the drawings were made were measured, so as to express in $\frac{1}{1000}$ths of an inch the transverse diameter of each root at the level of the apical cell or apical group. The result of the measurement is attached to each figure. Though this method of measurement is open to objection, in that it does not take account of the actual curve of surface of the apex of the root, still the results thus obtained give at least an approximate measure of the bulk of the roots. Further, as median longitudinal sections do not show any considerable variation of the actual curve of surface, this may be at least provisionally neglected. A comparison of these measurements, and of the meristematic structure of the apices in question, will show clearly that there is no strict and constant relation between them. Thus, of the three observed examples of a four-sided cell, one was $\frac{17}{1000}$ths of an inch in diameter, another $\frac{28}{1000}$ths, and a third $\frac{31}{1000}$ths. Conversely, figs. 3, 4, 5, 7, 8, 11, 16, and 18 are all from roots giving within narrow limits the same measurement. On the other hand, it is worthy of note that fig. 1 represents almost the smallest, and fig. 14 the most bulky of the roots investigated. Thus, my observations do not bear out in detail the idea which suggests itself from a comparison of the roots of the Polypodiaceæ with those of

the Marattiaciæ, but rather show the great irregularity of
the roots of Osmunda.[1]

Todea barbara.

For comparison with Osmunda regalis the roots of
Todea barbara were investigated with the following results :
Of a number of apices from which transverse sections were cut
not one showed a clearly-marked single apical cell. Some,
however, showed somewhat irregular arrangements, such as
that seen in fig. 23, in which case it appears uncertain whether
the meristem is referable to three or four initial cells. In a
majority of the roots observed it is clearly referable to four
initial cells (fig. 24), separated from one another by the four
principal walls (p).[2] The meristem in this case appears closely
similar to that described by Schwendener in the Marattiaceæ.
Longitudinal sections, however, do not show so close a cor-
respondence to the structure described by him in this group.
Pyramidal cells are not unfrequently to be found, as in fig. 25,
where two (x) are to be seen in a similar relative position to
those in the root of Osmunda represented in fig. 16. In
other examples, however, the initial cells have the form of
truncated pyramids, as in fig. 26, segments being cut off from
the truncated apex, as well as from the sides and base. This
arrangement probably corresponds to that shown in transverse
section in fig. 24.

The origin of the lateral roots was also observed in this
plant. As before, in Osmunda, the root originates from a
single cell of the endodermis, situated opposite one of the
groups of xylem. A few irregular divisions may also be traced
in adjoining cells of the cortex, but I was unable to ascertain
whether these cells take any active part in the formation of the
root-cap. Occasionally the divisions of the rhizogenic cell are
somewhat irregular, as in fig. 27, but usually the divisions are
symmetrical, as in figs. 28, 29. These two figures, however,

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[1] This subject will be more fully discussed below.
[2] I see no objection to using this term again here, though there are four of
them.

show that from the very first the initial cells of the young root are subject to a certain variation of form, being in some cases pyramidal, in others truncated. Thus, in this respect again, Todea barbara resembles Osmunda regalis.

Angiopteris evecta.

In the first place it may be stated that the comparatively few preparations of the apex of the root of Angiopteris which I have made, confirm the results of Schwendener's investigation. It would be interesting to know, as the result of a careful and detailed study, whether, under any circumstances, wedge-shaped initial cells, similar to those occasionally seen in Todea barbara, are to be found in Angiopteris. The descriptions of Russow[1] and of Holle[2] may have had some foundation in irregularities of this sort. In view of the inconstancy of form of the initial cells in Osmunda and Todea it would be rash to assert that pyramidal initial cells never occur in the Marattiaceæ, though cells of truncated pyramidal form appear to be typical in the roots of these plants.

Since the origin of the lateral roots of the Marattiaceæ has not hitherto been described, and as the material for this work was at hand, I have made some observations on this point. The lateral root of Angiopteris evecta takes its origin, as in other Ferns, from a single cell of the endodermis, situated opposite one of the numerous xylem groups (fig. 30). This rhizogenic cell enlarges, and divides repeatedly by walls perpendicular to the outer surface of the main root into a number of oblong cells, of which four lying at the centre of the group assume the properties of initial cells. In those cases which I have had under observation the walls of segmentation of the rhizogenic cell are not inclined to one another, and the initial cells are not pyramidal, but always oblong. The mode of further subdivision of the cells thus produced, and the position of the initial cells with their segments are clearly seen in fig. 31, taken from a more advanced lateral root than fig. 30. Fig. 32

[1] 'Vergl. Unters.,' pp. 107—109.
[2] 'Bot. Zeit.,' 1875, p. 301.

again shows the group of four initial cells, as seen in a transverse section through a young lateral root of about the same age as that in fig. 31. It is thus shown that the meristem of the lateral root of Angiopteris evecta assumes its definite character from the very first, and the four initial cells make themselves at once apparent. It is thus impossible to obtain evidence as to the origin of the mode of growth with a group of initial cells from the mode of their origin in the individual lateral root, since the character of the meristem is defined from the very first.

It is worthy of note that cells of the cortex lying around and outside the rhizogenic cell also undergo occasional and irregular division, but do not appear to play an important part. Other cells of the endodermis and cells of the pericambium also divide freely (fig. 30).

General Consideration of the Results.

The foregoing description of the structure of the meristem of the roots in Osmunda and Todea shows, in the first place, that there is no such strict uniformity in these plants as is found in the roots of Equisetum and the Polypodiaceæ, on the one hand, and, according to Schwendener, in the Marattiaceæ on the other. Secondly, the structure of the meristem, as above described in the Osmundaceæ, fluctuates in its characters between those two well-marked types, and affords numerous intermediate examples between them.[1] In order that those intermediate examples may be duly appreciated, it will be necessary to enter briefly upon the consideration of those two typical systems of construction between which the Osmundaceæ oscillate. The first, that typical of the Polypodiaceæ, is shown diagrammatically in fig. 33, which is quoted from Sachs ('Arbeiten,' Bd. ii, Taf. iv, fig. 12). Here the periclinal walls in the body of the root constitute an inter-

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[1] It may be objected that the roots observed may have been in a resting state, and so the apical cell may have been segmented as described by Sachs ('Arbeiten,' Bd. ii, p. 90). This, however, was not the case, as at least the large majority of the roots were in a state of active growth.

rupted series of confocal paraboloidal surfaces, their common
focus being situated in the apical cell itself. The periclinal
walls in the root-cap, in accordance with Sach's demonstration,
constitute a series of similar curves, which are, however, not
confocal, but are coaxial. Since the anticlinal walls cut the
periclinals at right angles, those in the body of the root, which
cut the confocal curves, present a concave surface to the axis
of growth, while those in the root-cap, cutting the coaxial
curves, present a convex surface (compare Sachs, l. c., Taf. iv,
fig. 11).

In the second type, according to the description given by
Schwendener, the arrangement differs from the above type in
certain important points, and a diagram may be drawn to show
the scheme of construction as in fig. 34. In this type of
structure there are in the first place walls in two radial planes,
which cut one another at right angles, and their line of inter-
section is the organic axis of the root. The periclinal walls
are none of them confocal, neither those which lie in the body
of the root, nor even those in the procambial cylinder; they
are, however, all coaxial, and their common axis is the line of
intersection of the radial walls, that is the organic axis. It
will be obvious that the periclinals are necessarily not confocal
in those organs where the apical cell (or the group of initial
cells) has the form of an inverted truncated pyramid, and gives
off segments from the truncated apex of the pyramid (the
lower end), which take part in the formation of the body of
the organ.[1] The apparently transverse walls by which succes-

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[1] In Sachs's 'Vorlesungen,' p. 556, the following passage is to be found
immediately succeeding an allusion to the apex of the root in the Marattiaceæ :
" Dagegen ist hervorzuheben, dass Scheitelzellen überhaupt nur dann im herge-
brachten Sinne möglich sind, wenn die Peri- und Antiklinen confokale Cur-
venschaaren darstellen oder kurz bei confokal gebauten Vegetationspunkten.
Ist der Vegetationspunkt dagegen mit fächerformig verlaufenden Antiklinen
durchzogen, ist die Volumenzunahme gegen den Scheitel hin am grössten, wie
oben bei fig. 288 gezeigt wurde, dann könnte man unter Umständen wohl
auch noch in erweitertem Sinne Scheitelzellen annehmen, allein die Schrift-
steller haben in diesen Fällen überhaupt nicht von Sheitelzellen geredet, und so
können auch wir davon absehen." In the scheme here constructed for the

sive segments are cut off from the lower ends of the truncated pyramidal initial cells, form part of successive periclinal curves. The number of periclinal curves may thus increase to a high figure; there is, however, no corresponding increase in the number of layers of cells in the mature root beyond a certain point; thus some at least of these periclinal curves must stop short, as would naturally be the case if the structure of the root were of the fan-like type, but maintained its cylindrical form. A second necessary consequence of the coaxial system of construction is that it would be impossible to trace the pro-cambial cylinder with a smooth outer surface up to the apical group, or in other words, that the pericambium could not be so traced as a continuous layer of cells, but would be composed of parts of successive layers. The same may or may not be the case for the epidermis, according as it is derived from portions of the lateral segments, cut off by an epidermal wall (as in the type described by Naegeli), or has a common origin with the root-cap from the cap-segments. Schwendener does not speak definitely on this point, but my own observations on Angiopteris indicate that the latter is the case. If it be so, then the epidermis must originate in a manner similar to that pseudo-epidermis described by Strasburger (' Conif and Gnet.,' Taf. xxiv, fig. 26) in Taxus baccata; it would originate from portions of successive layers of the meristem, not from one acropetally continuous layer. It is to be further noted in connection with the scheme represented in fig. 34, that the focus of each successive periclinal, by which segments are cut off from the initial cells to form the body of the root, lies at the time of segmentation at a point below the group of initial cells. In the former type, however (fig. 33), it will be readily seen that the focus or centre of construction lies in the apical cell itself (Sachs, *l. c.*).[1]   Thus in passing

root of the second type above described, we have a case of four initial o apical cells, giving rise to all the tissues of the organ, while the resulting tissues are arranged on a coaxial or fan-like system.

[1] It will be interesting to observe in this connection that Sachs has drawn attention to the fact that in Fucus the centre of construction lies outside

from the first to the second type of construction, there is a sinking or lowering of the centre of construction; this is still more apparent if we pass on to those roots in which the stratified structure (Sadebeck) is more pronounced. In these types the centre of construction is situated at a still lower point; since the anticlinals cut the periclinal curves at right angles, it follows that where the centre of construction is more depressed, the sides of the apical cell or of the initial cells will be less inclined to one another, and more nearly parallel than is the case where the focus lies at a higher point, for instance in the apical cell itself. The figures of Schwendener clearly demonstrate that this is actually the case in the Marattiaceæ, in which the initial cells appear almost oblong in longitudinal section. Lastly, it is clear that in our second type the initial cells may be represented to the mind as being gaps in the system of construction in just the same sense as the idea is applied by Sachs to the single apical cell.

Having now defined the two extreme types of construction, it remains to compare with them the structure of the apical meristem of the root in the Osmundaceæ, and especially in Osmunda regalis. The form of the apical or initial cells may first be taken into consideration. In some cases a single pyramidal apical cell has been found, as in fig. 1, but longitudinal sections always show (figs. 11, 12, 19) that even where the segmentation is most regular the cell has a narrower and deeper form than in the roots investigated by Naegeli and Leitgeb, that is, the lateral walls are less inclined to one another. This points to a depression of the centre of construction, though so long as the single apical cell maintains the pointed pyramidal form that point must be within the apical cell. In connection with these examples, which conform more nearly to the first type of construction, those must be mentioned in which two pyramidal cells are seen (fig. 16) which correspond not improbably to an arrangement like that in fig. 13, but cut in a plane oblique

the initial cells, and, indeed, outside the tissue of the plant altogether. This is exactly the reverse of what is found in the Marattiaceous type of root-structure.

to the radial wall (x).   Next may be taken the cell of the form
shown in fig. 20.   This is evidently a transition to the form of
the truncated pyramid, the wall adjoining Segment I being
deflected, so as to form at its lower part an oblique ending to
the whole cell.   Lastly, those examples may be cited in which
the initial cells have their lower ends (the apices of the pyra-
mids) decidedly truncated, as in figs. 17, 18, 21, 22.   These
figures, drawn from longitudinal sections, show a form of the
initial cells and arrangement of the segments which conforms
closely to the Marattiaceous type.   Thus, as regards the form
of the initial cells and the arrangement of the segments,
Osmunda provides various intermediate stages between the
two types of construction above described, and the transition
may be connected, as above pointed out, with a lowering of
the centre of construction.   Intermediate forms, though
with less gradual transitions, have been observed also in Todea
barbara, where both pointed and truncated initial cells have
been found.

In respect of the arrangement of the initial cells (where
more than one is present), as seen in the transverse section,
Osmunda again shows intermediate characters between the
two types.   From the three-sided cell, seen in fig. 1, we may
pass on to those arrangements with three initial cells seen in
figs. 6—8.   As I have pointed out above, these correspond
closely to the arrangement seen in transverse sections of roots
of the first type of construction at a point immediately below
the apical cell.   I would not suggest that the transition from the
meristem with a single three-sided pyramidal apical cell to this
with three initial cells could occur in the individual root, and
I have no evidence that it does; but, regarding the matter
from a phylogenetic point of view, we may well conclude that
in these examples we see the result of an upward continuation
of the principal walls in radial planes, so as to divide the apical
cell into three parts; in fact, this may be recognised as an in-
stance of filling up the gap in construction, accompanied, it
must be remembered, by a depression of the centre of construc-
tion.   It is hardly necessary to state that this meristematic

structure with three initial cells is closely related to that with four. If we remember that a four-sided apical cell has been observed in Osmunda, and, according to Holle ('Bot. Ztg.,' 1875, p. 301), also in Marattia cicutæfolia, a change similar to that above suggested in the case of the meristem with a three-sided cell, would in the case of a four-sided cell produce a structure corresponding to that observed by Schwendener in the Marattiaceæ, and by myself in Todea barbara. Here, instead of three radial walls meeting at a central point, there would be four such walls. Observation shows, however, that where this is the case the four walls do not meet exactly at one point, but slight irregularities are usually found. It is well known that in the roots of the type investigated by Naegeli and Leitgeb, the cap-segments divide by radial walls disposed crosswise into four cells; a similar arrangement has been shown to be the result of divisions in the root-cap of Osmunda (figs. 5, 7), even where the initial cells are of very different character. It can hardly be a matter of surprise that this incongruity between the radial walls of the root-cap and those of the body of the root is removed in that type of structure which appears to be the more permanent. In the Marattiaceous type the first formed radial walls, both in the root-cap and in the body of the root, are assimilated to one regular and uniform system, viz. two planes cutting one another at right angles. The incongruity of the radial walls in those examples of Osmunda in which there are three initial cells may be regarded as characteristic of a transitional structure, and it is not perpetuated in the more constant Marattiaceous type.

Before passing on to other points, a comparison is to be drawn between those roots of Osmunda with three initial cells and the structure of the apex of the stem of Lycopodium Selago, as shown by Strasburger ('Das Botanische Practicum,' figs. 93 and 94). Here there are also three initial cells disposed in a manner similar to that observed in the figures of Osmunda. Thus there are at least two examples now known of a type of grouping of initial cells, which is

omitted by Haberlandt in his 'Physiologische Pflanzen-Anatomie' (p. 45). This type might be intercalated between that in the stem of Selaginella Wallichii and that of the roots of the Marattiaceæ.

It has already been pointed out in connection with the Marattiaceous type of structure of the root that a necessary consequence of a coaxial but not confocal system of periclinal curves is that some of them at least must stop short if the root is to maintain its cylindrical form. A careful observation of median longitudinal sections of both Osmunda and Todea shows that a more or less abrupt termination of periclinal curves in a posterior direction, such as may be readily observed in root-caps, is to be found in these roots, not only in the root-cap but also in the tissues of the body of the root. Fig. 35, A and B, show examples from the cortex of Osmunda and Todea. In the former case, taken from the same root as appears in fig. 14, the arrangement of the cells strongly suggests that characteristic of the root-cap, that is a coaxial arrangement, no less than four periclinal curves terminating abruptly in a posterior direction within a comparatively short distance. In fig. B only one of the periclinal curves terminates within the short space represented. If further evidence be wanted, it may be found by referring to fig. 14, and observing that the anticlinals present a distinctly convex surface to the organic axis of the organ.

It was shown that a second necessary consequence of the coaxial arrangement is that the pericambium at the limit of the procambial cylinder would not be traceable as a continuous layer up to the initial group. Observations of both Osmunda and Todea show that this is actually the case; the pericambium originates, like the pseudo-epidermis of Taxus, from portions of successive layers of cells. It may here be again stated that it has not been possible to trace the epidermis as a continuous layer up to the initial group, nor have I been able to connect the origin of the epidermis with any wall, which appears in a definite position in each successive segment, as can be done in the type described by Naegeli and Leitgeb.

It has now been amply shown that the structure of the meristem in the roots of the Osmundaceæ possesses characters intermediate between the two types of construction above described, and that these characters are not constant even in roots from a single plant. The question remains whether there is variation in the individual root from time to time. Beyond some few cases of irregular structure, I have no evidence of any change of character of the meristem in the individual root; moreover, the observations on the origin of lateral roots show that in both Osmunda and Todea differences in the mode of segmentation of the rhizogenic cells may be found, which are quite as great as those observed in mature roots. Thus it is possible that the differences of structure observed in different roots may have had their origin in differences of the very first segmentation of the rhizogenic cells. I have, on the other hand, no reason for thinking that, within limits, variation of structure may not occur in the individual root.

It cannot escape observation that those roots in which the coaxial type of structure is most prominent the roots themselves are habitually more bulky than those in which the type of structure is confocal. Are these characters dependent one upon another? My observations lead me, as above stated, to the conclusion that as regards the roots of the species Osmunda regalis, differences of bulk of the roots cannot be correlated with corresponding differences of meristematic structure. Still, if we consider such a series of forms as Pteris, Osmunda, Todea, and Angiopteris, it certainly would appear that with an increase of bulk of the root there is also an advance in complexity of the meristematic structure, and a transition from the confocal to the coaxial type. Thus, a correlation which does not apply in detail for the individual species appears to hold for the roots of the above series of genera. As far as may be judged from observations at hand a similar progression may be traced roughly, but not in detail, for the meristem of the stem in different species of Selaginella.[1]

[1] Strasburger, 'Bot. Zeitg.,' 1873, p. 79, &c.; Sadebeck, 'Handbuch der

Sadebeck recognises among the investigated species two types of meristematic structure, the first, represented by S. serpens, S. Martensii, S. hortensis, S. viticulosa, has a wedge-shaped apical cell, the latter, represented by S. arborescens, S. Pervillei (= S. Vogelii, Baker), S. spinulosa, S. Lyalii (= S. lævigata, Baker), has a stratified character. Speaking generally, the members of the former series are trailing species, while those of the latter series of species are more robust. S. Wallichii, investigated by Strasburger, is also one of the more robust species, and has two wedge-shaped initial cells. Thus it appears that a greater complexity of meristematic structure is found also in the species of this genus to accompany a more robust and bulky character. But here again the correlation is not very close, though it is clearly recognisable, though this is not the case in the roots of the species O. regalis. On the ground of the above observations a general conclusion may be based which applies, at least for the plants above quoted, viz. that though greater bulk of the organ cannot be correlated with increased complexity of the meristem in the members of plants of the same species (Osmunda regalis), still that correlation can be traced in different species of the same genus (stem of species of Selaginella), and is clearly marked in the members of plants from different genera (roots of the series Pteris, Osmunda, Todea, and Angiopteris).

That the Marattiaceous type of structure of the apex of the root is an advance towards those types found among the higher vascular plants is recognised by every recent writer on the subject. It is interesting to note, however, that the approach is rather towards that structure which is found to be characteristic of the Gymnosperms, than to that of other vascular plants, or even other vascular Cryptogams, such as the Lycopodinæ. The coaxial mode of construction, which is dominant in the Marattiaceous type, is clearly represented also in the roots of the Gymnosperms, while the confocal type is more characteristic of the roots of Lycopodium and Isoetes.

Botanik' (Schenk), tom. i, p. 244, &c.; Treub, ' Recherches,' &c.; Bruchmann (ref.), ' Bot. Centrbl.,' 1884, No. 46.

Lastly, this peculiarity of structure of the roots is a character, among many others, which points out the Osmundaceæ as a family of Ferns having a close affinity with the Marattiaceæ. I have elsewhere shown[1] that, in the development of the leaf, and especially of the apex of the leaf, and in the conformation of the base of the leaf, the Osmundaceæ approach the Marattiaceæ; also that Todea is in certain characters nearer to them than Osmunda. The observations above detailed bear out this conclusion, and though such details should not be pressed too nearly home, still it should be noted that while no clear example of four initial cells was observed in Osmunda regalis, that structure appeared to be most frequent in Todea barbara. Again, the recent observations of Goebel (" Verleichende Entwickelungsgeschichte," 'Schenks' Handbuch,' Bd. iii, pp. 387—388) leave it still in doubt whether Osmunda be a Leptosporangiate Fern or not.[2] Thus in a number of characters, perhaps not very

[1] 'On the Comparative Morphology of the Leaf in the Vascular Cryptogams and Gymnosperms,' communicated to the Royal Society.

[2] My observations on the Sporangia of Osmunda, as far as they go, confirm those of Goebel; but better material was at hand for the investigation of the development of the sporangium in Todea barbara. Here the essential parts of the sporangium originate apparently from a single cell, which is, however, deeply sunk in the tissue of the young pinnule, but is exposed at the outer surface. It has a square base, that is the cell is not conical; divisions appear in it by walls perpendicular to the outer surface. When observed in surface view from the outside it is seen that four cells result from this division, three of them surrounding one central cell. This arrangement is not unlike that in the young sporangium of the Leptosporangiate Ferns; the chief difference is that up to this point the sporangium does not project far beyond the general surface of the leaf. Subsequently a periclinal wall separates a superficial cell from the archespore. The form of the archespore is a matter of some interest. Here, as in the case of the apical cell of the root, there is not exact uniformity; it is sometimes conical, sometimes rectangular. This variation depends upon the varying inclination of the three anticlinal walls, one to another; in some cases they are nearly parallel, and the result is that the archespore in these examples has a square base; in other cases they are inclined, and may meet one another below; the result is then a conical archespore. This appears to be the case also in Osmunda, judging from Goebel's figures (l. c., figs. 103A and B), and is no doubt to be connected with the

significant when considered individually, the Osmundaceæ are distinguished from the mass of the Leptosporangiate Ferns, and show themselves to be the nearest of all other groups of Ferns to the Marattiaceæ.

DESCRIPTION OF PLATES VIII & IX,

Illustrating Mr. F. O. Bower's memoir " On the Apex of the Root in Osmunda and Todea."

All the figures, excepting Figs. 9, 10, 15, and the diagrams, Figs. 33 and 34, were drawn with camera lucida, under objective c, Zeiss, ocular 4 ; but the drawings have been reduced by the lithographer to two thirds their original size ; the magnifying power is thus two thirds of 325, that is about × 216. In all cases the results have been controlled by observation under higher powers, viz. Zeiss's objectives D D, and, when necessary, F. The numbers attached to some of the figures show the diameter of the root at the level of the initial cells, measured in $\frac{1}{1000}$ths of an inch. The apical cell, or initial cells, are marked (x).

Osmunda regalis.—Figs. 1—8 and 11—22.

FIG. 1.—Slightly oblique transverse section, showing a three-sided apical cell with regular segments.

FIG. 2.—Four-sided apical cell, as seen in transverse section, with three segments.

FIG. 3.—Four-sided apical cell, with regular segments I—IV ; but segment V is cut off from the same side as IV. The dotted lines indicate the walls of

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fact that the sporangium is here more deeply seated in its early stages than in most other Ferns. That this is an intermediate form between that of the truly Leptosporangiate Ferns and that of the Marattiacea is obvious. In Angiopteris, Goebel has demonstrated an archespore with a square base (cf. 'Systematik,' p. 284, fig. 208), and such a form is only to be expected where the insertion of the sporangium is proportionately broad and the curvature of the outer surface slight.

Further details, together with figures, will be published later.

division in the root-cap. The section being a thick one, these walls could be seen by focussing deeply into the section.

Fig. 4.—Three-sided apical cell, segments less regular, and the apical cell itself is of comparatively small size. The square cell marked (x) may be assuming the function of an initial cell.

Fig. 5.—A. Section through the root-cap. B. Section from the same root immediately below A. B shows an irregular arrangement of the meristem, with apparently two initial cells (x).

Fig. 6.—Three initial cells, separated by principal walls, *p. p.* Sextant walls marked *s. s.*

Fig. 7.—A similar section immediately below the root-cap. By focussing deeply the wall represented by the dotted line appears; this is a division in the root-cap.

Fig. 8.—A similar section, but less regular.

Fig. 9.—Quoted from Naegeli and Leitgeb, Taf. xiv, fig. 5. A transverse section of the apex of the root of Pteris hastata, *s. w.*, as seen by focussing below the apical cell. *p. p.* = the principal walls. *s. s.* the sextant walls, which are in this case homodromous.

Fig. 10.—Quoted from Naegeli and Leitgeb, Taf. xii, fig. 8. Transverse section of the apex of a root of Equisetum hiemale, immediately below the apical cell. The sextant walls are here heterodromous.

Fig. 11.—Median longitudinal section, with pyramidal apical cell. *tr.* = cells developing as tracheides.

Fig. 12.—A similar section.

Fig. 13.—An irregular meristem: apparently two pyramidal initial cells of very unequal size.

Fig. 14.—Very irregular meristem, with a pyramidal apical cell (x). Segments undergoing repeated periclinal division. Note the curvature of the anticlinal or principal walls.

Fig. 15.—(× 370.) The products of the development of one segment, showing how one cell (x) takes the lead; the arrow shows the direction of the organic axis.

Fig. 16.—Section showing two pyramidal initial cells of equal size.

Fig. 17.—Two oblong initial cells; segments are cut off by periclinal walls from the lower end of them, as well as from the upper end and sides.

Fig. 18.—Two similar initial cells with segments.

Fig. 19.—A group of cells, derived by division from one rhizogenic cell of the endodermis. (x) The pyramidal initial cell of this very young lateral root.

Fig. 20.—Apical meristem from a young lateral root, which has extended to the outer surface of the cortex of the main root.

Fig. 21.—Young lateral root, with two oblong initial cells. *xy.* = the xylem of the main root. *p.* = pericambium.

Fig. 22.—A similar preparation of a young lateral root.

Todea barbara.—Figs. 23—29.

Fig. 23.—Transverse section, showing an irregular arrangement of the meristem.

Fig. 24.—A well-marked meristem in transverse section, showing four regular initial cells.

Fig. 25.—Longitudinal section, showing two pyramidal initial cells.

Fig. 26.—Longitudinal section, with two oblong initial cells.

Fig. 27.—Group of cells, derived by irregular division from one rhizogenic cell of the endodermis.

Fig. 28.—The same, but the division in this case is regular; two almost square initial cells.

Fig. 29.—Apical group, from a rather older lateral root, showing two pyramidal initial cells.

Angiopteris evecta.—Figs. 30—32.

Fig. 30.—Transverse section of a main root, showing the mode of origin of a lateral root from one cell of the endodermis (e. e.), also the xylem of the main root, and divisions in the pericambium (p.) and cortex (c.).   In the cells derived by division from the rhizogenic cell, two (x) may be recognised as the initial cells.

Fig. 31.—Group of cells of the lateral root, derived from a single rhizogenic cell : the oblong initial cells marked (x).

Fig. 32.—A section transverse to the axis of a very young lateral root, still embedded in the cortex of the main root.   It shows the four initial cells (x) as described by Schwendener.

Fig. 33.—Scheme of construction of the apex of a root with a three-sided pyramidal apical cell, quoted from Sachs's ' Arbeiten,' Bd. ii, Taf. iv, fig. 12.

Fig. 34.—Scheme of construction of a root of the Marattiaceous type.

Fig. 35.—A. A small portion of the young cortex of the same root of Osmunda regalis as is represented in Fig. 14. B. A small portion of the young cortex of Todea barbara.   The arrows point towards the apex of the roots.

Fig. 1

Fig. 3.
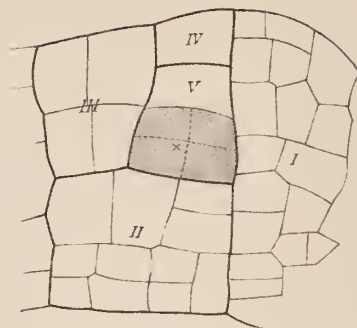
Fig. 2.
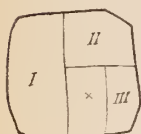
Fig. 7.
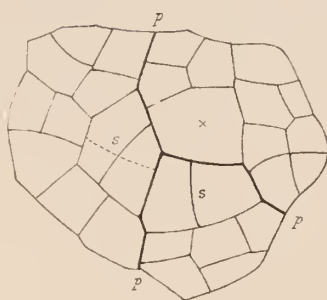
Fig. 8
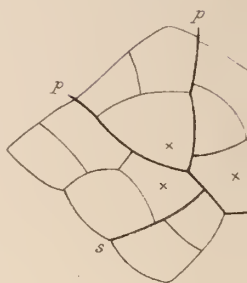
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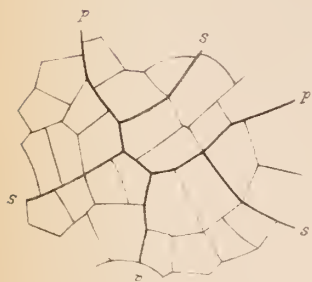
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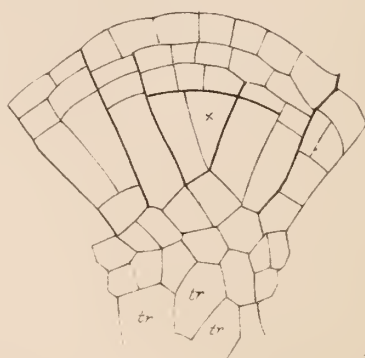
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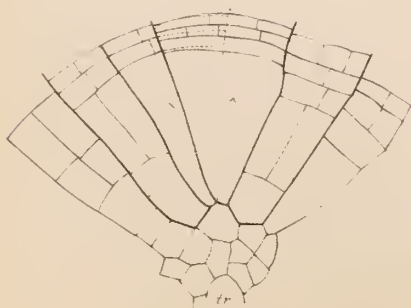
Fig. 13.
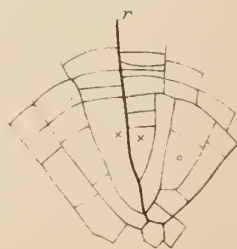
Fig 5 A

Fig. 5 B.
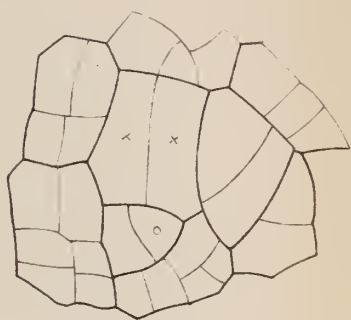
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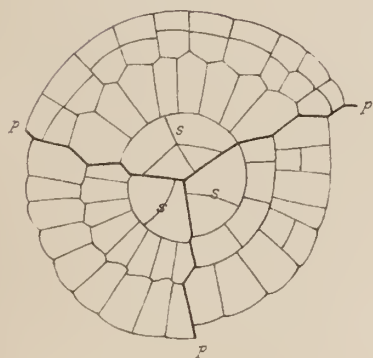
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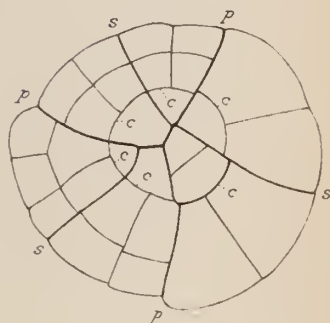
Fig. 14.

Fig. 15.

Fig. 17.
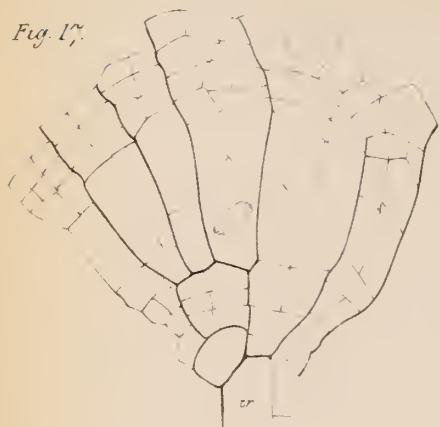
Fig. 18.

Fig. 19.

Fig. 20.
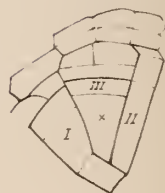
Fig. 24.

Fig. 25.

Fig. 26.
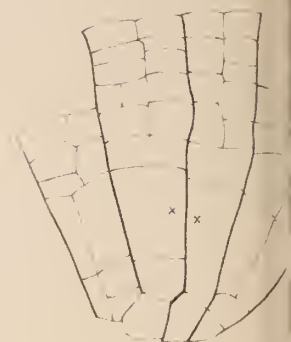
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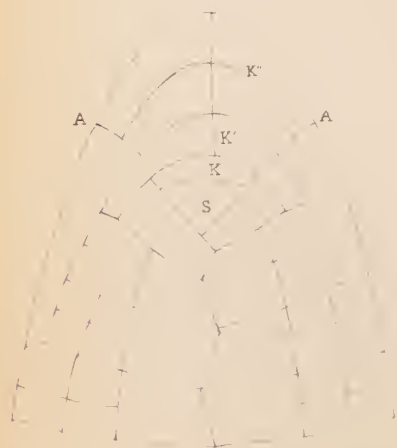
Fig. 34.
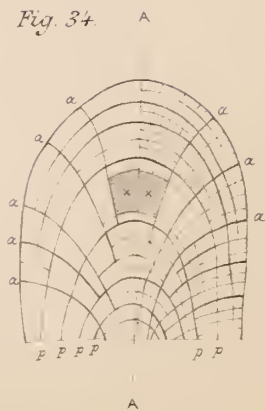
Fig. 35.

Fig. 21.
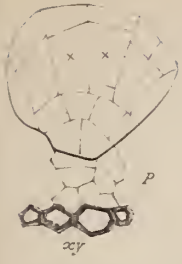
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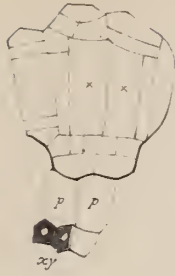
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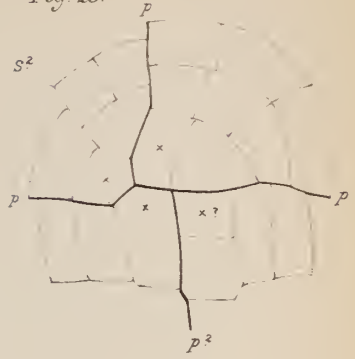
Fig. 27.

Fig. 28.

Fig. 29.

Fig. 31.
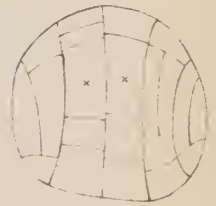
Fig. 30.
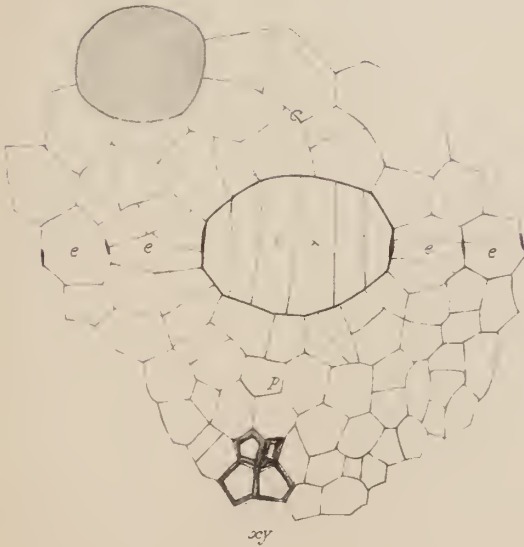
Fig. 32.
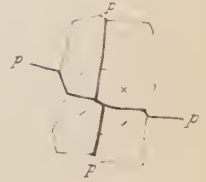
Fig 35 B.

Correction of an Error as to the Morphology of Welwitschia Mirabilis.

By

F. O. Bower.

At the conclusion of the second article on Welwitschia mirabilis communicated by me to this Journal (vol. xxi, New Series, 1881, p. 571), a postscript was appended, which suggested a possible explanation of the discrepancy between my observations of the process of development of this remarkable plant, and those of M. Naudin, who communicated an account of the germination of the same plant to the 'Gardener's Chronicle,' August 13th, 1881. The origin of the discrepancy was, however, soon after explained by a second letter from M. Naudin, published in the 'Gardener's Chronicle,' January 7th, 1882, which began as follows : " I find that by an accident, the seedling which I took to be that of Welwitschia, having sown the seed as such, and which I described in your columns (1881, vol. xvi, p. 217), is really not a Welwitschia at all ; all my Welwitschias (true) died, &c."

I had intended that this matter dealt with in my postscript should have died a natural death, and I should not have again returned to it, had it not been that in the reference to my article above quoted, which appears in the 'Botanischer Jahresbericht,' for 1881, Erste Abtheilung, Heft ii, p. 459, altogether undue prominence is given to the subject, by introducing it thus : " Das wichtigste in morphologischer Hinsicht bringt ein Postscriptum ;" whereas, when the facts are known, this is the least important part of the whole article.

Since there has been this misunderstanding, it may be well here to state briefly what the actual succession of members is

in the seedling of Welwitschia. In all the specimens which
I have examined, the following succession of members has been
observed : (1) two cotyledons, which are present in the mature
embryo ; (2) two plumular leaves decussating with the coty-
ledons, and capable of unlimited basal intercalary growth ; (3)
two structures, which appear between the plumular leaves, and
which I regard as buds in the axils of the cotyledons ; these
together form at least the great bulk of the crown ; (4) the
apical cone of the main axis, which remains rudimentary, and
bears no further appendicular organs. In all the plants at
Kew which have lived long enough, the cotyledons, after their
expansion during the early stages of germination, remain for a
considerable time persistent, without further increase in size
beyond a limit attained at an early period. Later they dry up,
and wither, leaving behind them dried tatters of tissue, like
those represented in 'Quart. Journ. Micr. Sci.,' 1881, pl. iii,
fig. 10. There are now living in Kew three plants of the
original set sown in August, 1880. Each of them has lost its
cotyledons in this way, but retains the ragged remnants of
them. The plumular leaves of these plants have grown to a
very considerable size, but are subject to progressive dis-
organisation of their tips, so that their present length (about
3 to 6 inches) does not represent the whole extent of their
previous growth. The two lobes of the crown (axillary buds)
have also grown to a considerable size, and have assumed the
appearance represented in 'Quart. Journ. Micr. Sci.,' 1881,
pl. xxxii, fig. 3.

Since all the plants germinated at Kew have developed in
the same way, and since the plant described by M. Naudin was
not a Welwitschia at all, the suggestion as to an alternative
mode of development is now entirely unnecessary, and may be
withdrawn. Such a suggestion would never have been made,
had not the observations communicated to the 'Gardener's
Chronicle' been contributed by a botanist of such standing as
M. Naudin. It may be now concluded that there is but one
type of development of Welwitschia mirabilis, and that
the type described by me from the plants grown at Kew.

E. Van Beneden's Researches on the Maturation and Fecundation of the Ovum.[1]

By

J. T. Cunningham,

Fellow of University College, Oxford; Superintendent of the Scottish Marine Station.

With Plate X.

A great many elaborate researches have been made in recent years into the series of phenomena in ova and spermatozoa which precede and accompany fecundation. By the synthesis of these it has been found that the series of phenomena is in almost all cases essentially the same; the more accurate the research, the more similar have the phenomena appeared in different animals. With regard to the ovum, the formation of polar globules is the most constant and most conspicuous event of its history previous to fecundation. Some years ago it was firmly established that there is a very great similarity between the changes which the germinal vesicle undergoes in the formation of polar globules, and the process of karyokinesis in cell-division.

At an earlier period it had been held that the germinal vesicle disappeared, and no connection had been surmised between that structure and the polar globules. These globules were regarded as unimportant drops of viscid substance expressed from the vitellus. In 1875 Bütschli[2] first described a spindle in an unfertilized ovum. It was observed in the egg of Cucullanus

[1] 'Arch. de Biologie,' 1883.
[2] 'Z. f. w. Z.,' vol. xxv.

elegans, and he was unable to prove that it gave rise to a polar globule, but he believed that the spindle and the extruded globule were identical, and that the spindle arose from the germinal spot. Bütschli's observations initiated a series of researches which threw a great deal of light on the history of the germinal vesicle. The most complete and trustworthy investigations were those of Fol and O. Hertwig; they declared that the polar globules were formed by a process identical with that of cell division and were equivalent to cells. Balfour, who never formed hasty conclusions, fully accepted this doctrine. Another result obtained by O. Hertwig was that the two pronuclei in the ovum, the male and female, united to form a single nucleus, before the commencement of the first segmentation.

The elaborate investigations of Van Beneden into the history of the ovum in Ascaris megalocephala have led to results which are in direct contradiction to the prevalent conception. He has satisfied himself (1) that the processes which accompany the formation of polar globules are fundamentally different from the processes of normal karyokinesis, that therefore the formation of polar globules is not a case of cell division; (2) that the two pronuclei do not unite together to form a single resting nucleus: what really takes place is that each pronucleus behaves as a nucleus about to divide, and that the chromatic V shaped loops and achromatic fibrils formed in each contribute to form a single karyokinetic figure. In these two points then Van Beneden is in opposition to those authors who have hitherto been regarded as most authoritative. He has made a complete study of the subject with what seems to be eminently favorable material, and gives a detailed account of several steps which have before been left in some obscurity. It is therefore important as well as extremely interesting to examine his figures and descriptions and to enquire, firstly, if his conclusions are well founded, and, secondly, what relation his results bear to our knowledge of the life-history of the cell and of reproduction.

Material.—Ascaris megalocephala is a Nematode of

considerable size (average 25 cm. in length), found in large numbers in the intestines of horses. It can easily be obtained, especially in winter (at Liège), in the living condition in any numbers required. The preparation of its ova is effected with great ease : its spermatozoa are large, non-motile, and conspicuous; lastly, from a single female a large number of ova in each successive stage can be obtained, from those still in the ovary to those which have begun to segment. In order to expose the sexual organs all that is needed is to slit the animal longitudinally with scissors. The maturation and fecundation of the ovum take place during its slow passage down the oviduct and uterus, the latter of which contains large numbers of spermatozoa. A given stage of development occurs at a certain position in the sexual tube, so that by taking a small portion of the tube thousands of eggs are obtained all in the same stage. The uterus is fifteen to twenty cm. in length, and the ova come into contact with the spermatozoa at its upper end. With regard to the penetration of the male element the author remarks, ' Il est facile de démontrer en cinque minutes, non pas seulement à des histologistes expérimentés, mais au dernier des profanes, la pénétration du zoosperme.

Methods.—1. By examining the eggs fresh, either in Kronecker's artificial serum (distilled water 100, NaCl 6, HNaO ·06) or in the liquid of the body of the Ascaris, all the phases of the penetration of the spermatozoon can be made out : in the oldest ova the various membranes, the two polar globules, and the pronuclei can be seen, but the details of the changes cannot be made out in fresh eggs.

2. One of the methods which gave the best permanent preparations was to kill the ova with nitric acid 3 per cent., leave them in this for one hour, then wash with water, place one or two hours in alcohol 33 per cent., then transfer to alcohol 70 per cent. For younger ova it is necessary to open the uterus and prepare the ova on the slide; for the ova at the inferior end of the uterus this is not necessary. For staining after the use of nitric acid, borax carmine, fuchsine, and methyl green gave the best results.

3. Fix with alcohol 33 per cent. only, transfer to 70 per cent., stain with picrocarmine or borax carmine, mount in glycerine or balsam.

4. Osmic acid, picrocarmine; mount in picrocarminated glycerine. This method does not serve for eggs with complete envelopes.

5. Glacial acetic acid may be used for fixing, but is not recommended.

6. For the oldest eggs the author found at the end of his researches that the only method was to place living females in weak alcohol. The older eggs with thick membranes go on developing for a certain time, till, after some months, the alcohol kills them, and then fine preparations, showing the pronuclei and the early stages of segmentation, are obtained by staining with picrocarmine and mounting in glycerine.

I. First Period of the Maturation of the Ovum.

This stage includes the development of the ovum in the ovary and its changes in its passage down the oviduct, until, arrived at the upper part of the uterus, it is ready to receive the spermatozoon. The author has given a great deal of attention and considerable space in his memoir to the structure of the ovum, on account of the interest attached to the question—Can the position of the embryo be recognised in the ovum even before segmentation? Some indication has been given by recent researches of Van Beneden himself and others that this question may be answered in the affirmative; that in the ovum of a bilateral animal a right and left side can be found corresponding to the right and left side of the embryo, and similarly an anterior and posterior end. The author found two years previously that in the ovum of Corella parallelogramma, a simple Ascidian, from the first phases of segmentation, the median plane of the embryo, its anterior and posterior ends were fixed. Facts of a similar kind have

been made known by Rabl,[1] Hatschek,[2] Roux,[3] and Rauber.[4]

The eggs at the lower end of the oviduct, in the condition in which they receive the spermatozoon, take, when liberated on a slide in an indifferent liquid, the form of an ovoid. There is little difference between the length of the longer axis of the ovoid and the shorter. In the ovum a morphological axis can be distinguished with dissimilar poles; this axis coincides with one of the shorter axes of the ovoid. At one of its poles there is a slight projection of the substance of the ovum; this projection is limited by a circular outline, and it is to the centre of the projection that the spermatozoon attaches itself. This pole of the axis may therefore be named the pole of impregnation, while the opposite end is called the neutral pole. In order to see well the structure of the ovum it must be examined a little before it has reached the lower end of the oviduct. The slightly projecting portion may be called the polar disc. The portion of the vitellus beneath the disc is more sombre than the rest, and is called the central medulla. This is enveloped, inferiorly, by a layer of the vitellus, a little more transparent, which reaches the surface of the ovum in a zone all round the polar disc, distinguished as the parapolar region. This second layer of the vitellus is the intermediate layer. Finally enveloping the intermediate layer in its lower portion is a cortical layer of vitellus, the most transparent of all, which extends to the surface of the ovum from the neutral pole to the limit of the parapolar region. This limit between the cortical layer and the parapolar region, is the parapolar circle. The germinal vesicle lies in the intermediate layer close to the inner surface of the cortical layer.

When the ovum leaves the rhachis of the ovary it has the form of a mallet; the extremity of the handle of the mallet is

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[1] 'Jena. Zeit.,' Bd. x.

[2] 'Arb. Zool. Inst., Wien,' Bd. iii.

[3] 'Zeit. der bestimmung der hauptrichtungen des Freschembryos,' Leipzig, 1883.

[4] 'Zool. Anz.,' 1883, No. 147.

the region at which the polar disc is formed. Nearly the whole of the handle of the mallet is occupied by the intermediate layer, and its surface is the parapolar region. The ovum assumes the ovoid form by the gradual shortening of the handle of the mallet and the consequent decrease in extent of the parapolar region.

When the polar disc first appears it has some affinity for carmine : it exhibits a striation perpendicular to its surface, and contains none of the deuteroplasmic elements. After a time in the polar disc two layers can be distinguished : the superficial is not stained by picrocarmine. This achromophilous layer collects towards the centre of the disc, and there the chromophilous layer thins out, and finally disappears. Meantime the vitelline membrane forms as an extremely thin layer all over the ovum, including the polar disc, but not over the achromophilous layer in its centre. This mass remains bare, occupying an aperture in the vitelline membrane—the micropyle, from which it usually projects slightly. This projecting mass of clear protoplasm van Beneden calls the plug of impregnation.

The vitellus contains deuteroplasmic elements of three kinds, which can be distinguished after treatment with osmic acid and picrocarmine. They are (1) hyaline spheres, (2) homogeneous droplets, and (3) refringent corpuscles.

The hyaline spheres are definite in outline, and slightly tinged with the carmine; they show no structure; within them are usually smaller spheres like vacuoles.

The homogeneous droplets are of indefinite outline and irregular shape, and are probably spaces in the protoplasm of the vitellus, filled with fluid or semifluid substance.

The refringent corpuscles are definite in outline, somewhat angular in shape, and highly refractive. They are often arranged in series, and contribute to give the vitellus a radiate striation, the series converging towards the centre of the ovum.

The protoplasm forms a continuous thin external layer round the vitellus, and fills up the spaces between the formed elements.

Examined with $\frac{1}{18}$ homogeneous immersion of Zeiss it is seen to be finely punctated, and the points are united by slender threads.   The protoplasm is in fact, as has been discovered by many histologists in animal cells, made up of a reticulum of moniliform threads.   There must be an interfibrillar substance filling up the meshes of the reticulum.

Nothing is said upon the question whether the ovum is attached to the rhachis by the end of the tail of the mallet, or by some other point.   This is a question of some importance, because in some animals, e.g. Lamellibranchs, the micropyle is formed by the pedicle of attachment of the ovum.

Vitelline Membrane.—In the ova, already detached, taken from the lower part of the ovary, there is no vitelline membrane, so that in this animal the formation of the micropyle is not due to the presence of a pedicle of attachment. Meissner[1] has stated that in the nematode egg studied by him, the micropyle was formed in the way indicated, while other authors have denied the existence of any membrane before the entrance of the spermatozoon.   In Ascaris megalocephala, although it is usually impossible to detach a membrane before the entrance of the male element, it is certain that the external layer of the vitellus has before that period become differentiated and resistant.   Ova, when ready to receive the spermatozoon, do not flatten out in all directions as they do at an earlier stage, but the vitellus escapes at the pole of impregnation.   Immediately after a spermatozoon has attached itself to the plug of impregnation a membrane is present which becomes separated from the parapolar region and the polar disc during preparation. In unfertilized females a membrane is formed round the ovum. It may be concluded then that the vitelline membrane is not formed in consequence of fecundation.

The Germinal Vesicle, whose position in the ovum has already been indicated, appears in the fresh state as a clear space in the vitellus containing a dark spot, the germinal spot or nucleolus.

In ova treated with osmic acid and picro-carmine (see fig. 1, Pl.

[1] 'Z. f. w. Z..' Bd. v, 1853.

X), the germinal vesicle is very conspicuous; it is coloured uniformly pink, is of spheroidal form, and apparently homogeneous. The germinal spot is a brilliant deeply-stained spherical globule, with a regular contour. It is situated near the periphery of the vesicle, and is surrounded by a spherical mass of substance distinct from the rest of the vesicle. This envelope of the germinal spot is more deeply stained than the rest of the vesicle, and remains distinct throughout the changes of the latter. It is named by van Beneden the prothyalosoma, the rest of the vesicle being called the accessory portion. Nearly always in the accessory portion there are one or two formations similar to the prothyalosoma, each containing a "pseudo-nucleolus;" these disappear during the changes which the vesicle undergoes.

The prothyalosoma projects slightly beyond the general surface of the germinal vesicle. Round the vesicle there is a distinct membrane: the layer within this occasionally shows minute granulations. In preparations obtained by treatment with nitric acid and borax-carmine the germinal spot appears not as a single body but as a group of small globules. The author believes that the germinal spot is always composed of two discs in close apposition, each disc consisting of four globules. The globules are connected by a substance with less affinity for staining substances than the globules themselves.

In ova perfectly ripe for fertilization treated with alcohol and borax carmine, the nucleolus appears as a group of numerous globules in which no definite arrangement is seen. The accessory portion presents a punctated appearance, and the small granules to which this is due are arranged in rows resembling moniliform threads of protoplasm. These rows of granules are chiefly derived from the transformation of the membrane. Similar rows of granules connected by very fine threads are also to be seen in the prothyalosoma.

In ova which are perfectly ripe, whether treated with nitric acid or alcohol, the spherical shape of the vesicle is deformed by the intrusion of a homogeneous droplet on each side so that the vesicle appears in optical section in the form of a T.

The result then of the author's study of the germinal vesicle before fecundation is as follows.

Before the ovum is quite mature, that is to say, some little time before it has reached the upper part of the uterus, the vesicle is spherical and bounded by a thin achromatic membrane. As maturation progresses traces of a spindle of achromatic fibrils are observed in the neighbourhood of the germinal spot; the fibrils are situated in the prothyalosoma, and are continuous with the chromatic discs of the spot. Nothing is to be seen resembling the convoluted chromatic filaments which appear in nuclei about to divide. The action of picro-carmine shows that chromatin is diffused to a slight extent throughout the vesicle. The pseudo-nucleoli behave towards staining fluids somewhat otherwise than the germinal spot. The germinal spot is in contact with the interior of the membrane of the vesicle. The accessory portion is composed of a denser substance next to the membrane and an internal more fluid part. Towards the time of the entrance of the spermatozoon some of the fluid contents passes out from the vesicle, and it therefore loses its rotundity and becomes smaller. Gradually both the membrane and the cortical layer of the accessory portion resolve themselves into delicate moniliform threads similar to those of the protoplasm of the vitellus. A similar change takes place in the substance of the prothyalosoma, but a little later. The vesicle is altered in shape by the intrusion of vitelline elements, and appears T shaped. This is the whole extent of the changes which take place before the entrance of the spermatozoon (see fig. 2, Pl. X. Into the ovum represented a spermatozoon has already penetrated; but the condition of the germinal vesicle shown may be reached before the contact of the male element).

The author gives some details concerning the ova of unfertilized females, but he says nothing about the formation of polar globules in such ova which is the most interesting point.

Spermatozoa in the Uterus.—Spermatozoa are found in numbers in all parts of the uterine epithelium; but they occur in greatest abundance in the upper extremity of the tube where the ova first meet with them. Since the uterus is dis-

tended with ova, the spermatozoa would be carried down by the descent of these if they were not protected in the deep furrows and depressions of the uterine epithelium. A large number in excess of those which effect an entrance into ova are carried down mechanically, but as these unattached spermatazoa diminish in number towards the lower end of the uterus, they probably pass up again along the epithelium by means of their amœboid movements.

Structure of Spermatozoon.—The ripe spermatozoon consists of two parts:

(1) A head of hemispherical shape enclosing a nucleus composed of chromatin only. Round the nucleus is a perinuclear layer finely punctated and with no distinct boundary, and external to this is a cortical layer composed of a reticulum of moniliform threads.

(2) A tail which may have one of various shapes. The tail is enveloped by a membrane which ends in a free border where the head and tail join, the head being destitute of membrane. The substance of the tail is iu the young state entirely protoplasmic, but in older spermatozoa contains a refringent body of larger or smaller size; a layer of protoplasm persists between the membrane and the refringent body.

The structure of the protoplasm of the head is easily seen, and the author takes occasion to point out that, according to researches of his own, the fibril of a striated muscular fibre has a structure similar to that of the moniliform fibrils of this protoplasm. He thinks it probable that such a structure is common to all protoplasm.

The spermatozoa when first introduced into the female organs are spherical in shape and completely destitute of refringent body. One half of the sphere is covered by a layer of substance in the form of a cup which is more homogeneous than the rest of the sphere. This cup grows out into the form of a papilla, and increasing in size gives rise to the tail. The first stage of its growth gives the spermatozoon a pyriform shape; then by further elongation of the tail the whole appears like a bell suspended by a string; this is the campanuliform

stage.   The interior of the tail is formed by a prolongation of the cortical layer of the protoplasm of the head; in this internal protoplasm at the campanuliform stage the refringent body begins to develop; it is at first linear.   At the lower end of the refringent body is a plate of homogeneous substance still more refringent, called the limiting plate.   Finally, by the increase in thickness of the refringent body the shape of the spermatozoon becomes conoid.   It is at this stage that the membrane becomes distinctly visible.   The head of the spermatozoon can throw out amoeboid processes.   The head uncovered by membrane corresponds to the plug of impregnation in the ovum, while the external protoplasm of the tail corresponds to the cortical layer of the vitellus.   The spermatozoon can effect fertilization either at the pyriform, campanuliform, or conoid stage (see Plate X, fig. 3).

II.—Penetration of the Spermatozoon or Copulation of the Sexual Elements.

The penetration of a spermatozoon into the vitellus does not in itself, as is now well known, constitute fecundation.   In many ova, as in the present case, a great many processes take place after the entrance of the spermatozoon, before the formation of the segmentation nucleus.   In other cases the period between the two events is shorter, the polar globules being ejected before the spermatozoon enters.   Van Beneden, therefore, distinguishes the two events as copulation of the sexual elements, and actual fecundation.

Notwithstanding the attention which has been paid to the phenomena of fecundation recently, these researches of Van Beneden are the first which have traced the history of the spermatozoon in a Nematode ovum.   Bütschli[1] and Auerbach[1] were the last investigators who concerned themselves with the reproduction of Nematodes, and they obtained no information as to the fate of the spermatozoon after its contact with the ovum.   So recently as 1883, A. Schneider,[1] who specially

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[1] For the earlier literature, vide Balfour, 'Comp. Emb.,' vol. i.

studied Ascaris megalocephala, denies the existence of a male pronucleus; he states that the spermatozoon is entirely absorbed by the vitellus, and that the two pronuclei are formed by the division of the germinal vesicle.

In the immense majority of cases only one spermatozoon penetrates into the vitellus. Having examined preparations which Prof. Van Beneden kindly sent me, I am convinced of the perfect accuracy of what he says on this point.

In the earliest stages a spermatozoon is seen fixed by its head to the plug of impregnation; in the next stage half immersed in the vitellus, in the next completely immersed. There is no uncertainty as to the identification of the sperma- tozoon ; its size enables it to be seen with a low power, its refringent body distinguishes it from all the elements of the vitellus. Before entering an ovum a spermatozoon fixes car- mine only in its nucleus, the moment it attaches itself to the plug of impregnation all its protoplasm is affected by the staining solution. Thus it is possible, without the evidence afforded by focussing, to distinguish immediately between a spermatozoon simply attached accidentally to the surface of the ovum and one actually in the interior of the vitellus.

In one or two instances two spermatozoa were seen within the vitellus ; but the question whether both of them form male pro- nuclei which unite with the female pronucleus was not decided.

The author points out that it has not yet been proved for all animals that fertilization is effected normally by a single sper- matozoon. In Echinoderms, Fol and Hertwig have shown that only a single spermatozoon enters the ovum. In Ascaris megalocephala and Petromyzon,[1] according to Calberla, the micropyle is closed by the spermatozoon itself. Kupffer and Benecke,[2] on the other hand, describe the entrance of several spermatozoa into the ovum of Petromyzon ; and there are numerous published researches to support either side of the controversy. But it has never been proved in the ovum of any animal that the formation of more than one male pronucleus

[1] Calberla, ' Z. f. w. Z.,' Bd. xxx.
[2] ' Vorgang der Befruchtung im Ei von Petromyzon,' Königsberg, 1878.

is a normal occurrence.   Fol has shown that in Asterias two
male pronuclei are sometimes formed, and give rise to ab-
normal segmentation and abnormal larvæ.

In prepared eggs of Ascaris megalocephala, in which a
spermatozoon is attached to the plug of impregnation, a space
is often seen between the vitelline membrane and the vitellus
in the region round the micropyle.   This lifting of the mem-
brane never occurs at later stages, or at other parts of the
vitellus.   The author discusses very fully the literature on the
subject of micropyles, pointing out that in very few cases has
it been proved that the micropyle, when it exists, forms the
exclusive means of entrance for the spermatozoon.   For the
bibliography of this subject the reader may refer to Van
Beneden's work, and to Balfour, ' Comp. Embryology,' vol. i.
Van Beneden is inclined to believe that every ovum has a
morphological axis, at one end of which a single spermatozoon
enters ; but he does not maintain that this has yet been
proved.

After the spermatozoon has fixed itself to the plug of im-
pregnation (the two elements uniting by their homologous
poles), the latter descends towards the interior of the vitellus,
taking the spermatozoon with it.   The head of the spermatozoon
executes amœboid movements, which aid its penetration.   When
the head has passed the micropyle, the membrane covering
the tail unites with the vitelline membrane so as to close the
aperture ; and the complete membrane is called the oosper-
matic envelope.   As the spermatozoon advances into the in-
terior of the vitellus the membrane follows it, so that when it
is entirely immersed there is a very slight prominence to show
where it entered.   After the spermatozoon has entered, its
protoplasm becomes less granular ; its nucleus is less refrin-
gent and less deeply stained.   Apparently some of the chro-
matin of the nucleus diffuses through the protoplasm of the
head.

After the spermatozoon is completely immersed it is impos-
sible to distinguish either plug of impregnation or polar disc.
The penetration of the spermatozoon is effected, at least in part,

by the contraction of the protoplasm of the plug of impregnation, the fibrils of which run into the interior of the egg.

III.—Formation of Polar Globules.

Here again the author gives a critical review of the literature, of which the chief points are : that polar globules are formed in vegetable ova ; that in Toxopneustes lividus they are formed before the ovum leaves the ovary ; that, according to Calberla, they are formed in Petromyzon, at the metamorphosis of the Ammocœtes ; that in Ascidians the germinal vesicle disappears in the ovary, and polar globules have not been found in their ova ; and lastly, that in Vertebrata true polar globules have never been discovered. .

The view that the polar globules are cells is supported by the division of the first globule which was observed by O. Hertwig, and still more by the discovery of Trinchese[1] and Blochmann[2] that in this division the phases of karyokinesis are again exhibited.

The stage which previous researches left most obscure was the transformation of the germinal vesicle into the so-called directive spindle. Trinchese and Blochmann state that the equatorial plate is derived from the nucleolus of the ovum. Trinchese considers the achromatic spindle as the vesicle transformed, while Blochmann gives no opinion on the point. In Vertebrata, except by Hoffmann in Teleosteans, nothing resembling a directive spindle has ever been found.

Formation of first Polar Globule.—The structure from which the first polar globule is formed and which is itself derived from the germinal vesicle, Van Beneden calls the Ypsiliform figure, because it resembles a Greek upsilon Υ.

The figure consists of three branches, each composed of achromatic fibrils, and of a number of chromatic globules at the point of union of the three branches, together with certain other elements all achromatic. The figure, as its name implies,

[1] 'Acad. dei Lincei,' t. 7.
[2] 'Z. f. w. Z.,' Bd. xxxvi.

is bilaterally symmetrical : two of its branches which meet at an obtuse angle are similar to one another, the third is dissimilar. The plane of symmetry passes through the axis of this third branch.

At the outer pole of each of the similar branches is a large group of granules. The form of the whole group is somewhat hemispherical, the flat base being towards the branch. From the hemisphere pass outwards a few diverging radii of moniliform protoplasm, part of the protoplasmic reticulum of the ovum. The fibrils of each branch arise from the base of the hemispherical group of granules ; some of these fibrils are regular in thickness, some present varicosities, some are moniliform. Two or three fibrils in each branch are conspicuous by their greater thickness, they lie near the axis of the branch and are inserted into the chromatic globules. The other fibrils are curved, and change direction when they reach a clear spherical body, in the centre of which the chromatic globules are placed. The axial fibrils penetrate the spherical body. The fibrils which are near the vertical branch of the Y meet in acute angles and interlace, and in this way the vertical branch is formed. The vertical branch is not columnar but flattened in a plane perpendicular to the line uniting the two poles of the similar branches. Another illustration which the author employs to facilitate the conception of the figure is that of the toy known as cup-and-ball. The stem of the cup is formed by the vertical branch of the Y, the ball is the clear spherical body containing the chromatic globules, while at opposite points the cup runs out into two poles : it must be remembered that the stem of the cup is flat, not round. The limits of the clear spherical body become less distinct as development advances. Usually in optical section there are two groups of chromatic globules, one on either side of the plane of symmetry ; each group shows two globules but really contains four. Outside the figure in each of the angles between the vertical branch and the superior branches is a mass of deuteroplasmic vitelline substance limited externally by a convex surface (see fig. 4, Pl. X).

Relation of the Ypsiliform figure to the Germinal Vesicle.

A comparison between figs. 2 and 4 shows what this relation is. The clear spherical body is the prothyalosoma, the chromatic globules are the germinal spot or nucleolus. The spheroidal masses at the sides of the figure are the vitelline elements seen at an earlier stage to push before them the walls of the germinal vesicle. The axial fibrils of the similar branches are formed in the substance of the prothyalosoma partly. The remaining portions of the figure arise from the transformation of the accessory portion of the germinal vesicle, and especially from its membrane.

In this connection the author points out that it is doubtful whether nuclear membranes and nucleoli are always homologous. He regards chromatin as a kind of fluid which is absorbed by the more solid achromatic substance. An achromatic membrane is not equivalent to a chromatic one, and while in the ovum all the chromatin or nearly all is contained in the germinal spot, in an ordinary cell it is diffused through various parts of the nucleus. Hence he believes the germinal corpuscle is not homologous with the nucleolus of a cell. The polar stars of the figure appear early in the transformation of the vesicle, at first close to the prothyalosoma from which they afterwards separate. The author regards them as centres of attraction. The pseudonucleoli probably go to form achromatic fibrils. The prothyalosoma is at first covered by achromatic fibrils above, but these separate and it is then exposed.

Formation of Polar Globule from Ypsiliform Figure.

The figure passes gradually to the periphery of the ovum; there is reason to believe that it reaches the surface at the pole opposite to that at which the micropyle existed, as in one case the spermatozoon was still attached to the oospermatic mem-

brane when the figure had reached the surface at the opposite pole.

The two poles of the similar branches both reach the surface of the vitellus.   The angle between these branches now becomes more and more obtuse, until the prothyalosoma reaches the surface of the ovum.

The groups of achromatic granules at the poles of the similar branches become, by the fusion of the granules, homogeneous discs.   A similar fusion takes place among both the axial and peripheral achromatic fibrils of the figure; at the same time the whole figure becomes smaller.   By this time the spermatozoon has passed to the centre of the ovum, and fibrils are seen going from it to reach by a curved course the vertical branch of the Y.   The vitellus has now lost all appearance of formed elements except its protoplasmic reticulum, and is very transparent; but after this commences a formation of granules round the spermatozoon, which gradually extends throughout the vitellus till the whole is granular and somewhat opaque, and the protoplasmic reticulum is obscured.

The next change in the ypsiliform figure is that its vertical branch becomes superficial, still remaining at right angles to the line connecting the poles of the similar branches; and then a new branch is formed opposite to the original vertical branch, so that a cross is formed at the surface of the vitellus with the prothyalosoma in its centre.   The two last-formed branches of the cross undergo a longitudinal cleavage which seems to play an important part in the liberation of the polar globule.

After this all the branches of the cross disappear, and nothing remains but a finely granular disc where the cross existed, surrounded by the granular vitellus.   The periphery of the disc is distinctly defined, and in its centre are the prothyalosoma and its contained chromatic elements unchanged. Later even the limits of the disc become obscure.   The prothyalosoma is somewhat reduced in size.

The polar globule is formed by division of the prothyalosoma and its contained globules in a plane tangential to the surface

of the vitellus (see ·fig. 5, Pl. X). It is evident from what has been said that this plane is one which at an earlier stage would have passed through the axis of the spindle formed by the similar branches of the Y. On this point Van Beneden insists strongly, that the plane of division as observed by him passes through the axis of the spindle, and not, as described by other observers, through the equator. Each of the chromatic discs divides into two, one half going into the polar globule; thus the chromatic part of the latter also consists of two discs. In the polar globule there is very little, if anything else, besides half the prothyalosoma.

During the formation of the polar globule a thick layer of homogeneous substance is thrown off from the vitellus and lines the oospermatic membrane; this is the first perivitelline envelope. The polar globule remains attached to the internal surface of this envelope.

The half of the prothyalosoma which remains within the vitellus is called the deuthyalosoma (i. e. deuterohyalosoma); it contains of course two chromatic discs, or two groups of chromatic globules.

Changes in the Spermatozoon during this Period. —The tail gradually becomes smaller, and its refringent body diminishes and finally disappears. In one female the refringent body was observed first to have separated from the spermatozoon, and at last to have escaped from the vitellus altogether and to lie between it and the perivitelline envelope. This, however, is exceptional; the refringent body seems usually to be absorbed by the vitellus. Ultimately the spermatozoon in the centre of the ovum consists of a chromatic globule round which is a clear perinuclear layer, itself surrounded by a layer of granular or reticulated protoplasm.

Formation of the Second Polar Globule.—Immediately after the expulsion of the first polar globule appears in the vitellus a second complicated figure, whose development culminates in the formation of the second polar globule. The elements which enter into the composition of this figure are:

(1) The deuterohyalosoma and its two chromatic discs.

(2) The surrounding protoplasm, which has become scarcely distinguishable from the vitellus.

Each of the chromatic discs gives off a filament, formed, during the division of the disc, at the expulsion of the first polar globule. Similar filaments appear at the internal ends of the discs. The two discs next divide up into smaller chromatic globules and the filaments become more numerous— in what way is not clear. Thus a spindle is formed. The deuterohyalosoma increases in size.

The spindle is directed radially, one of its poles being deep the other superficial. At each of these two poles, outside the deuterohyalosoma, appears a star, consisting of a group of achromatic granules surrounded by radiating moniliform threads. These stars may be—at least in part—formed from the substance of the achromatic fibrils of the first figure, but the continuity cannot be traced. The rays of the stars directed towards the deuterohyalosoma elongate and meet, while the remainder disappear to a great extent. Thus a figure in the form of a lozenge is produced, one of whose diagonals is occupied by the spindle within the deuterohyalosoma (see fig. 6, Pl. X).

One half of the lozenge now gradually disappears, and thus a figure is formed similar to the ypsiliform figure of the earlier period. The deep pole of the axial spindle now approaches the surface of the vitellus, and the division of the deuterohyalosoma takes place in a plane passing through the axis of the spindle, not in the equatorial plane.

The second pseudokaryokinetic figure, as Van Beneden calls it, is, in its later stages, complicated. An examination of the Plate XVIII *bis*, and of the text referring to it, shows that the division of the deuterohyalosoma into two parts—one of which is to form the second polar globule, the other the female pronucleus—that this division takes place long before the second polar globule is expelled from the vitellus. Before the change in the position of the spindle occurs, the spindle and the deuterohyalosoma are divided axially by a median plate, which moves through an angle so as to become superficial, and thus the globule is expelled (see figs. 7 and 8, Pl. X).

As before, each half of the deuterohyalosoma contains two
plates of chromatin, each composed of four globules.

Even before the second polar globule is expelled, the female
pronucleus has, in place of a distinct membrane, a periphery
of punctations. At the time of the expulsion the achromatic
fibrils outside the divided deuterohyalosoma disappear. The
author believes that the second polar globule is the exact
equivalent of the female pronucleus, and that the membrane
round it is an achromatic nuclear membrane.

Changes in the Vitellus during the Formation of
the Second Polar Globule.—The external layer of the
vitellus becomes first clear and reticulated in structure, and
then becomes separated from the vitellus to form the second
perivitelline envelope. After its separation the vitellus con-
tracts, and a perivitelline space is produced. In ova prepared
with alcohol the second envelope becomes fluid and cannot be
distinguished from the perivitelline space. The second polar
globule remains attached to the surface of the vitellus. At
the period of the expulsion of the second globule, as at that of
the first, two concentric circles appear on the surface of the
vitellus round the polar body. The outer one divides the
vitellus into two parts, one of which contains the female
pronucleus, the other the spermatozoon.

No important change occurs in the spermatozoon during
this period; often a radial striation of the vitellus is visible
round the male element.

Formation of the Pronuclei.—The expulsion of polar
globules is quite independent of the presence of the spermato-
zoon. In the rabbit the first polar globule is formed while
the ovum is still in the ovary, and the membrane formed after
this, known as the vitelline membrane, is to be regarded as
the homologue of the first perivitelline envelope in Ascaris.
These processes take place in the rabbit even when spermatozoa
are prevented from reaching the uterus. The influence of the
spermatozoon on the ovum begins with the conjugation of the
pronuclei.

In the living female the ova do not develop further than

the formation of the pronuclei : the later stages were studied in eggs which had continued to develop after the females were placed in alcohol.

The female pronucleus at first contains two chromatic discs, which are, as has been seen, probably derived directly from the two discs of the germinal spot. After each division the chromatic discs regain their size. Each disc is really made up of four smaller globules united by a substance composed of moniliform threads. The acromatic membrane of the pronucleus is also composed of very delicate moniliform threads, and still more minute fibrils unite the membrane to the chromatic elements. The changes which the pronucleus undergoes are exactly similar to those of a cell nucleus about to divide. The chromatin in the discs diffuses along the achromatic fibrils, and ultimately is found in fibrils at the surface of the pronucleus, the central part being almost empty except for scattered threads. The pronucleus is usually divided by a partition into two parts. The male pronucleus is formed from the chromatic elements in the centre of the spermatozoon and the perinuclear layer : the chromatic layer outside this is thrown off. A membrane appears round the perinuclear layer and then the same changes take place as in the female pronucleus (see fig. 9, Pl. X).

The female pronucleus approaches the male : the chromatin in each comes to form a single thick sinuous fibril which breaks up into two ∨-shaped loops. The four loops arrange themselves as a star, on each side of which are achromatic fibrils forming a karyokinetic spindle. At each pole of the spindle appears a star derived from the vitellus. The loops at the equator now divide longitudinally, and the closed ends of the two groups so produced diverge from one another. As the groups diverge achromatic fibrils appear connecting them : these are produced from the loops as they divide, and are not parts of the original spindle as has been supposed by other authors. Hence Flemming's notion that the chromatic loops travel along the achromatic fibrils to the poles. As was described in cells by Pfitzner the chromatic loops have a moni-

liform structure. The divergence of the chromatic groups is due to the contraction of the achromatic fibrils towards the polar stars. These stars take no part in the edification of the daughter nuclei which are formed by the gradual diffusion of the chromatin through the achromatic substance. No convolution stage occurs in the reconstruction. The mature daughter nuclei have the same structure as the mature pronuclei (see figs. 11, 12, 13, Pl. X).

Flemming observed a longitudinal division of the chromatic loops in cells but could not prove that this division was concerned in the division of the equatorial plate formed by the loops. Van Beneden has proved that the latter phenomenon is due to the former. It has struck me that here lies the solution of the controversy between Strasburger and Flemming pointed out in a previous essay.[1] The division of chromatic elements in the equatorial plane, affirmed by Strasburger and denied by Flemming, is probably the same thing as the longitudinal division of the chromatic loops described by the latter.

The division of the vitellus into two blastomeres is not due to a complete constriction. A furrow appears on the outside of the vitellus, but division is completed by a " cell-plate " such as that described by Strasburger in plants. This cell-plate is a homogeneous layer formed from the intermediate achromatic fibrils and in its centre a limiting surface appears which separates the two blastomeres. The rest of the intermediate fibrils are assimilated by the vitellus.

The first two blastomeres divide in exactly the same way. Only four chromatic loops are present at the equator of each spindle.

Theory of Fecundation.—Of the four chromatic loops in the first segmentation-spindle two are male and two female. Similarly every cell formed from the two first blastomeres, and each of these blastomeres themselves, is hermaphrodite. Therefore ovarian cells destined to become ova are originally hermaphrodite, and likewise the cells of the testis destined to become spermatozoa. But an ovarian cell in becoming an

[1] This Journal, 1882.

ovum throws off certain parts of its nucleus. It is very plausible to suppose that the part of the nucleus thus eliminated is the male part, and the ovum after the expulsion of polar globules thus differs essentially from an ordinary cell. Van Beneden therefore calls the ovum containing a female pronucleus a gonocyte.

In the development of spermatozoa also, some part is thrown off—the cytophore or blastophore, described in very many different animals. In Ascaris megalocephala a spermatogonium divides into two cells—the spermatogemmæ; each of which divides into four cells—the spermatocytes, which become spermatozoa. Between the four spermatocytes is the cytophore, composed of four parts, one derived from each spermatocyte. Each part of the cytophore contains an element which takes up carmine, but whether it is derived from the nucleus of the spermatocyte Van Beneden does not know. In a subsequent memoir,[1] of which I have only been able to see an abstract, Van Beneden and Julin have discovered a globule thrown off by the spermatogonium of Ascaris megalocephala before the cytophor is formed. They believe this globule to be expelled from the nucleus in the same way as described by Van Beneden in the ovum, and to be equivalent to a polar globule; the cytophore is not formed by a process equivalent to cell division. Van Beneden then concludes that the spermatozoon has lost the female part of its nucleus, and is a male gonocyte. The two gonocytes unite to form a complete hermaphrodite cell.

With regard to the opposition between himself and previous investigators, Van Beneden points out that the discrepancies cannot be due to his methods or interpretation, because in the segmentation of the ovum his results confirm and extend those of earlier researches. If he recognised normal karyokinesis in the one case he was able to do so in the other if it existed.

[1] 'Bull. Acad. Roy. Belg.,' vii, 1884, p. 312.

Critical Remarks on Van Beneden's and other
Researches.

In spite of the conclusion arrived at by O. Hertwig and Fol,
that the polar globules were formed by a process equivalent to
cell-division, we find on examining their works that in some
animals certain stages of the process as described by them do
not agree with the corresponding stages of karyokinesis. In
karyokinesis it has been clearly shown that the whole nucleus
is transformed into the achromatic spindle and its chromatic
loops. If we study plate ii of Fol's elaborate memoir[1] we
find that in Asterias glacialis only a portion of the ger-
minal vesicle or spot is employed in the formation of the
" Amphiaster de rebut;" the figure is represented as originating
entirely on the outside of the germinal vesicle. It is evident
from that plate that a large portion of the germinal vesicle and
spot is left to disappear in the vitellus. On the other hand,
if we examine plate viii of the same work we find that in
Pterotrachea the germinal vesicle and spot are entirely trans-
formed into the spindle of the amphiaster and its equatorial
plate. The results of Fol on other animals are not very
decisive on this point (see figs. 15, 16, Pl. X).

If we turn now to O. Hertwig we find that his account of
the phenomena in Hirudinea[2] supports the direct transfor-
mation view. On the other hand, his account of the same
phenomena in Asteracanthion (pls. vi and viii of his paper in
'Morph. Jahrb.,' Bd. iv) agree with the results of Fol on
Asterias glacialis with the following difference:—He found
that a certain portion of the germinal spot passed into the
centre of a star, which was forming in a portion of vitelline
protoplasm projecting into the interior of the germinal
vesicle (see figs. 14, 18, Pl. X). It is possible also
that part of the substance of the germinal vesicle goes
to form the spindle, but Hertwig is not very decisive on
this point. It seems, then, that in Echinoderms the germinal
vesicle and spot are not directly but only partially transformed

[1] 'Commencement de l'hénogénie,' Geneva, 1879.
'Morph. Jahrb.,' Bd. iii.

into the "Amphiaster de rebut," or "directive spindle." The formation of the directive spindle from the germinal vesicle is not fully described in the works previous to Van Beneden's. On this point Van Beneden is very clear and decided, and so far his account agrees with the descriptions of karyokinesis. But in his description of the structure of the figure produced and the formation of a polar globule from it, he is directly at variance with previous writers, and the process he describes is quite different from karyokinetic division. Fol and Hertwig describe and figure these later stages as identical, except in very minor points such as the length of the chromatic elements, with the division of cell nuclei, and they give the same account for very different animals; e. g. Echinoderms, Molluscs, Leeches. It follows, then, either that O. Hertwig and others were mistaken, or that Van Beneden is wrong, or that the processes in Ascaris megalocephala are fundamentally different from those in other animals. Van Beneden himself mentions two very recent publications which support the theory of the cellular nature of polar globules. One is by Trinchese,[1] the other by Blochmann,[2] and they have shown that the division of the first polar globule after its expulsion which had been observed by O. Hertwig, again exhibits the phases of karyokinesis. It will naturally be expected that ultimately the process of formation of polar globules will be proved to be essentially the same in all animals, but future researches must decide on this point. As is well known, a directive spindle has been sought in vain in the ova of Vertebrates, and the expelled elements in these are not globular in form. Hoffmann[3] alone has described an Amphiaster de rebut in Teleostean ova, but has not traced its history.

Several papers have appeared subsequently to Van Beneden's memoir, on the subject of fecundation, &c. P. Hallez,[4] in March, 1884, published a note of researches on Ascaris

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[1] 'Mem. Acad. Lincei,' tom. 7.
[2] 'Z. f. w. Z.,' Bd. xxxvi.
[3] 'Natuurk. Verh. Koninkl. Acad. Deel.,' xxi.
[4] 'Comptes rendus,' No. 11, tom. 96, 1884.

megalocephala. The results he gives would have been interesting ten years ago, but at the present time they are scarcely deserving of serious attention. The formation of "the" polar globule is mentioned but not described. The male pronucleus is said to be a spindle with rods at its equator. The female pronucleus is described as a magnificent star. There is no micropyle. His account of the development of spermatozoa is astonishing; the young spermatozoa he calls deutospermatoblasts; these congregate in pairs, and when they separate each gives out a corpuscle de rebut resembling a polar globule. The rest of the development takes place within the female organs. M. Hallez has been original enough to mistake the refringent body for the spermatozoon itself. The nucleus is always, he says, outside the body of the spermatozoon.

A general essay on fertilization is given by A. Sabatier.[1] He finds that three kinds of globules are expelled from the ovum, precocious globules, "globules tardifs," and true polar globules. These eliminated parts are the male element; in the spermatozoa it is the central part of the original cell which is eliminated. Sabatier sought to prove that in the parthenogenetic ova of Aphides polar globules were not formed, but was not quite successful. He refers on this point to Weissmann's account of parthenogenesis in Daphnoidea.

Another paper on the subject which treats of Ascaris megalocephala, is by Prof. Moritz Nussbaum,[2] of Bonn. In some points he confirms Van Beneden, in others is at variance with him. His figures and descriptions of the directive spindle are very different from Van Beneden's and far less complete. He confirms Van Beneden's results as to the presence of four chromatic loops in the first segmentation spindle. His conclusion with regard to the theory of fecundation is that it consists in the union of two homologous cells.

Bütschli,[3] in discussing the essential meaning of fecundation,

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[1] 'Revue des Sciences Naturelles,' iii, 1884, p. 362.
[2] 'Arch. f. Mik. Anat.,' vol. xxiii.
[3] 'Biol. Centralbl.,' iv, 1884.

starts with the process of sexual multiplication in the colonial Volvocineæ. He does not agree with the theory which regards polar globules as the eliminated male element, pointing out that in the lower Algæ there is no such elimination in the sexual conjugation. In this connection I am led to refer to the most suggestive remarks on the origin of sexual reproduction in a paper by P. Geddes[1] on the cell-theory. Geddes regards the original life of a cell as a cycle passing from the encysted stage to the ciliated, then to the amœboid and then to the plasmodial. In the last stage the amœbæ unite and the union results in increased activity of the cells which is shown by their greater power of motion and especially in their more rapid multiplication. From this plasmodial union, exemplified in Myxomycetes, he derives the sexual union of a male and female cell. He points out that a multiple union exists according to Gabriel in Actinosphæria, according to Gruber in Gregarines, and results in reproduction. That the nucleus is not indispensable in conjugation results he thinks from the demonstration by Gruber[2] that in the young Actinophrys a nucleus is really absent and develops independently in adult life; and even if the essence of reproduction lies in the union of nuclei rather than in that of the protoplasm, we must suppose on the evolution theory a primitive stage in which nuclei had not yet been developed. All this, however, although giving a common meaning to phenomena at first sight isolated, does not explain why the nucleus when it does exist plays such a conspicuous part in fecundation and exhibits such a universal and striking process as the formation of polar globules. We require yet a series of patient and skilful researches like these of Van Beneden into the phenomena in question in all living forms, to give us accurate and more extensive data from which to generalise. It is not ten years since Bütschli first discovered the directive spindle, and ten years more will probably throw

[1] "A Restatement of the Cell-theory," 'Proc. Roy. Soc. Edinb.,' vol. xii, 1883-4.
[2] 'Zool. Anzeiger,' No. 118, 1882.

further light on the inner meaning of reproduction, the most mysterious and most distinctive phenomenon of life.

EXPLANATION OF PLATE X,

Illustrating Mr. Cunningham's report on "Van Beneden's Researches on the Maturation and Fecundation of the Ovum."

List of reference letters.

a. p. Accessory portion of germinal vesicle.   *c. z.* Cortical layer of vitellus. *dth.* Deuterhyalosoma.   *h. d.* Homogeneous droplets of vitellus.   *h. s.* Hyaline spheres of vitellus.   *p. d.* Polar disc.   *p. g.* First polar globule.   *p. g'.* Second polar globule.   *p. i.* Plug of impregnation.   *pth.* Prothyalosoma. *r. sp.* Refringent body of spermatozoon.   *r. g.* Refringent granules of vitellus. *s. n.* Secondary nucleus of germinal vesicle.   *sp.* Spermatozoon.

FIG. 1.—Ovum before the entrance of the spermatozoon. Osmic acid, picro-carmine. Obj. 8, Hartnack. (V. B., Pl. X, fig. 5.)

FIG. 2.—Deformation of the germinal vesicle at the time of the penetration of spermatozoon. Alcohol, borax-carmine. Obj. 8, Hartnack. (V. B., Pl. XIV, fig. 10.)

FIG. 3.—Spermatozoon in the uterus, conoid stage. Obj. 8, Hartnack. (V. B., Pl. XI, fig. 14.)

FIG. 4.—Ypsiliform figure. Nitric acid, borax-carmine. Obj. $\frac{1}{12}$th, Zeiss. (V. B., Pl. XV, fig. 3.)

FIG. 5.—Expulsion of first polar globule. Nitric acid, borax-carmine. Obj. $\frac{1}{12}$th, Zeiss. (V. B., Pl. XVI, fig. 17.)

FIG. 6.—Second pseudokaryokinetic figure. Nitric acid. Obj. $\frac{1}{12}$th, Zeiss. (V. B., Pl. XVII, fig. 12.)

FIG. 7.—Later stage of same. Alcohol. (V. B., Pl. XVIII *bis*, fig. 1.)

FIG. 8.—Formation of second polar globule. Alcohol. (V. B., Pl. XVIII *bis*, fig. 3.)

FIG. 9.—The two pronuclei. Alcohol, borax-carmine. Obj. $\frac{1}{12}$th, Zeiss. (V. B., Pl. XIX *bis*, fig. 9.)

FIG. 10.—Longitudinal division of chromatic loops in first segmentation. Alcohol, borax-carmine. Obj. D, Zeiss. (V. B., Pl. XIX *bis*, fig. 25.)

Fig. 11.—Same stage, showing achromatic part of the nuclear structure. Same method. (V. B., Pl. XIX *ter*, fig. 3.)

Fig. 12.—Later stage. (V. B., Pl. XIX *ter*, fig. 10.)

Fig. 13.—Edification of the daughter nuclei. (V. B., Pl. XIX *ter*, fig. 13.)

Fig. 14.—Ovum of Nephelis three quarters of an hour after deposition. Formation of first polar globule. Acetic acid, 1 per cent. glycerine. Zeiss, F, oc. 2. (O. Hertwig, 'Morph. Jahrb.,' Bd. iii, Pl. ii, fig. 3.)

Fig. 15.—Formation of "Amphiaster de rebut" in ovum of Asterias glacialis. *No.* Germinal vesicle, *no.* Germinal spot. *Ar'.* Amphiaster de rebut. *V.* Vitellus. Picric acid, glycerine. 400 diam. (Fol, 'Comm. de l'Hénogénie,' Pl. ii, fig. 3.)

Fig. 16.—Formation of "Amphiaster de rebut" in ovum of Pterotrachea. Acetic acid, glycerine with alcohol. 400 diam. *F.* Central. *F'.* Peripheral fibrils of forming spindle. *f.* External rays of polar sun. *a.* Centre of polar sun. *Nov.* Envelope of germinal vesicle. *V.* Vitellus. (Fol, ibidem, Pl. vii, fig. 14.)

Fig. 17.—Formation of first polar globule in Pterotrachea. Acetic acid, glycerine. *ai.* Aster interne. *Cr'.* Corpuscle de rebut. *Fc.* Chromatin granules. *Ar'.* Chromatin left in ovum. *Ev.* Envelope. (Fol, ibidem, Pl. viii, fig. 5.)

Fig. 18.—Origin of directive spindle in ovum of Asteracanthion. Osmic acid, picro-carmine. Thirty minutes after deposition. (O. Hertwig, 'Morph. Jahrb.,' Bd. iv, Pl. viii, fig. 1.)

Fig. 19.—Ovum of Asteracanthion, forty-five minutes after deposition. Acetic acid. (Ditto, ditto, fig. 3.)

Fig. 20.—Ovum of Mytilus, immediately after deposition. *a.* Directive spindle. *b.* Residue of germinal vesicle. (Ditto, ditto, Pl. x, fig. 2.)

Fig. 21.—Ovum of Ascaris megalocephala. Second directive spindle. *K.* Refringent body of spermatozoon. *Sp. K.* Head of spermatozoon. *IRK.* First polar globule. *II. Rs.* Second directive spindle. *I.* First. *II.* Second vitelline envelope. (Nussbaum, 'Arch. f. Mik. Anat.,' Bd. xxiii, Pl. x, fig. 34.)

Figs. 22 and 23.—Two spermatogemmæ, each composed of four spermatocytes and showing the cytophore in the centre in four pieces. Osmic acid, picro-carmine. (V. B., Pl. XIX *ter*, figs. 19 and 20.)
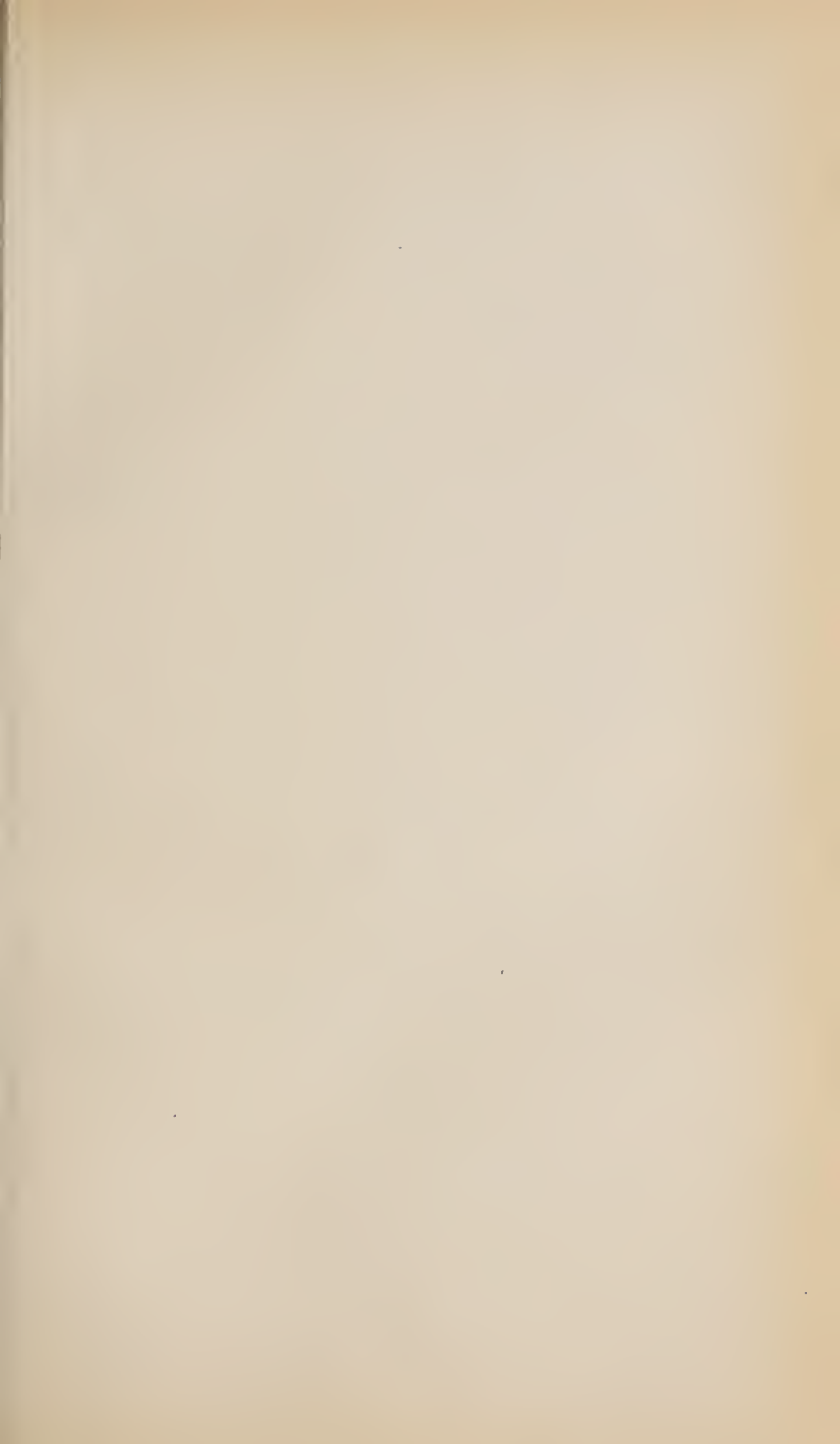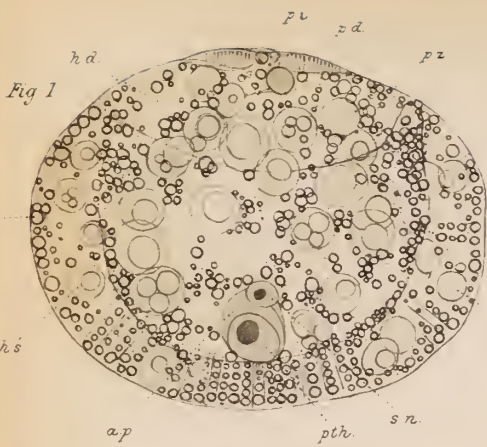
Fig 1.
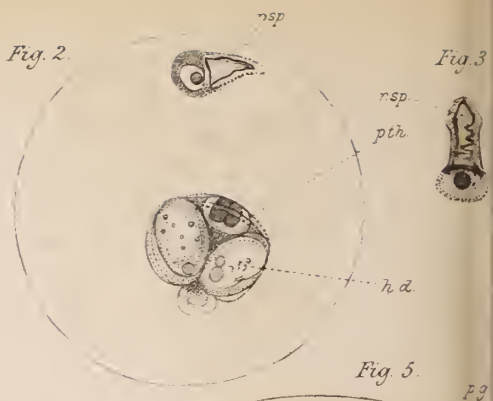
Fig. 2.

Fig.3.

Fig. 4.

Fig. 5.
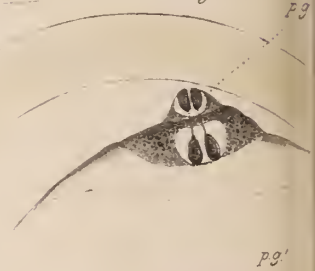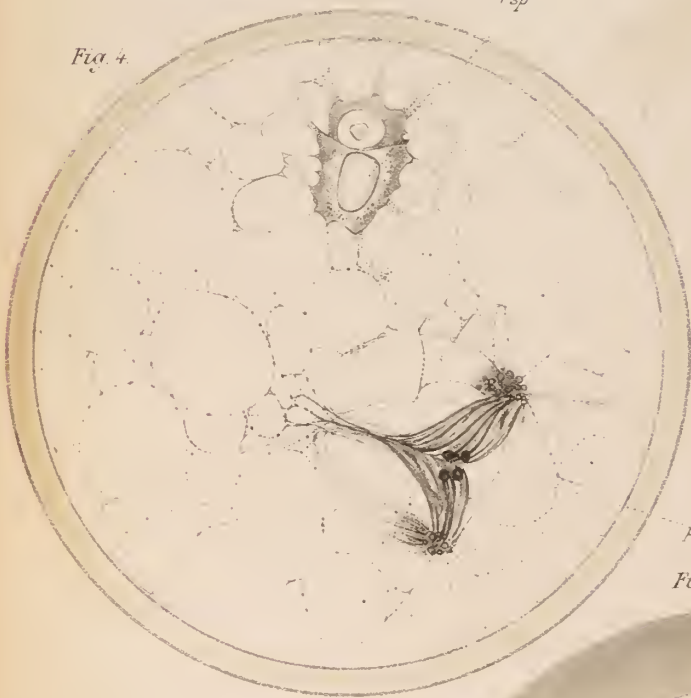
Fig. 6.

Fig 7.

Fig. 8.
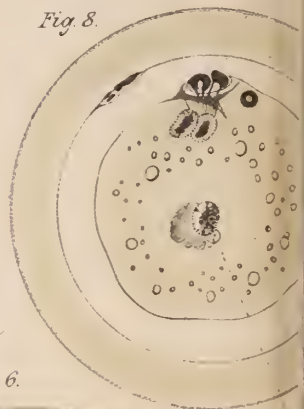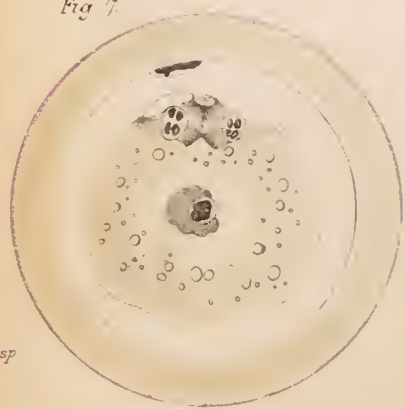
Fig 16
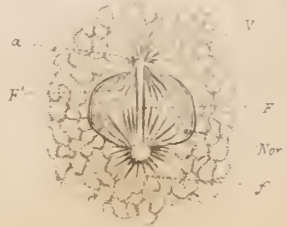
Fig. 20.

Fig. 9.
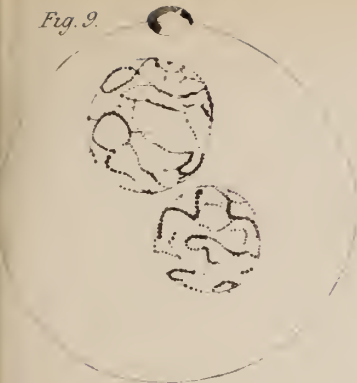
Fig. 10.
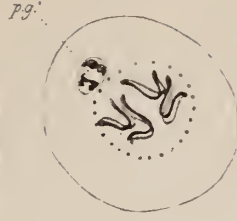
Fig. 11.

Fig. 12.

Fig. 13
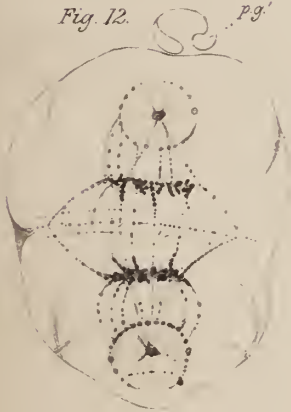
Fig. 15.
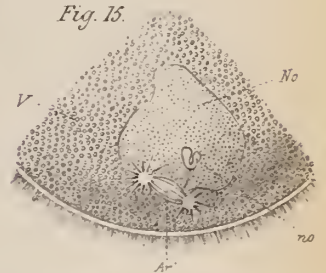
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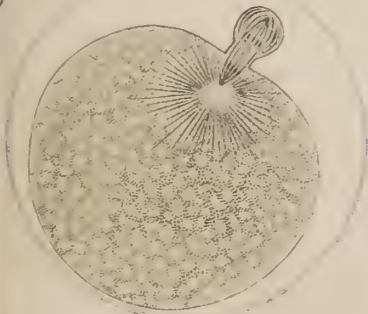
Fig. 18.
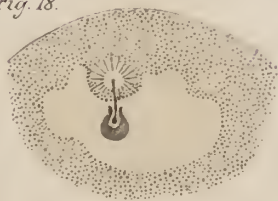
Fig. 19.
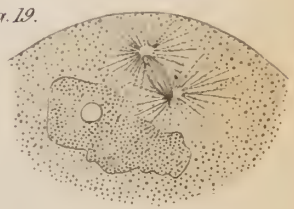
Fig. 21.
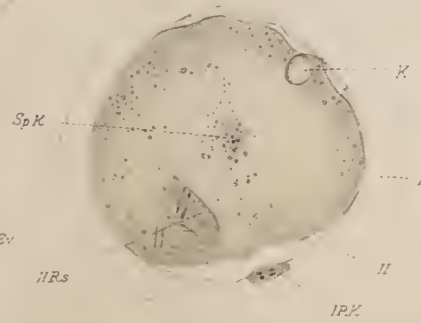
Fig. 22.

Fig. 17.

Fig. 23.
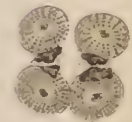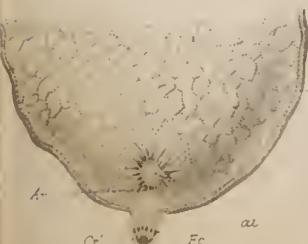
On the Suprarenal Bodies of Vertebrata.

By

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With Plates XI and XII.

THE suprarenal bodies of Vertebrates are, as is well known,
made up of two sets of elements, sharply distinguished from
one another, both by their adult structure, and by their mode
of origin in the embryo. The substance which from its posi-
tion in the mammalian suprarenal is known as "medullary" is
now almost universally admitted to consist of metamorphosed
nerve-cells, which arise from one or more of the ganglia of
the sympathetic system. As to the origin of the remainder,
however, the so-called "cortical" substance, little is certainly
known. In Elasmobranchs, Balfour[1] describes the homologue
of this substance as "making its appearance . . . as a rod-like
aggregate of mesoblast cells, rather more closely packed than
their neighbours, between the two kidneys near their hinder
ends;" but he leaves it an open question, whether these cells
arise from the general indifferent mesoblast surrounding them,
or whether they are derived from any of the adjacent organs
of the embryo.

These observations of Balfour were followed, in 1882, by two

[1] "Elasmobranch Fishes," p. 246.

important papers by Braun[1] and Mitsukuri,[2] the one dealing with the development of the suprarenals in lizards, the other in mammals.

In lizards, Braun describes the cortical substance as arising "as a thickening in the walls of the vena cava inferior." In the earliest stage figured by him, a large mass of cortical blastema is already established, as seen in Pl. 1, fig. 4 of his paper. In this figure, as in all the others given by Dr. Braun, it is noticeable, as he himself says, that "the flattened, nucleated endothelium (of the blood-vessel) is easily to be distinguished" from the adjacent tissue, and that it shows no sign of proliferation. It is therefore difficult to conclude from this account that the suprarenals arise as appendages of the blood-vessels themselves, Braun's observations throwing little more light upon the real origin of the cortical substance than did the earlier ones of Balfour.

In the same way Mitsukuri, treating of mammals, finds the first rudiment of the cortical substance in a little knot of isolated mesoblast cells "on each side of and ventral to the aorta, on the inner side of the Wolffian bodies, and dorsal to the mesentery."

Gottschau, in a later paper[3] has described in mammals phenomena nearly in accordance with those observed in lizards by Braun,—emphasising more than Mitsukuri the connection between the cortical substance and the adjacent blood-vessels.

From none of these observations can we learn anything of the mode of origin of the blastema described, each author taking up its history at a point when the cells composing it have already lost any connection which they may primitively have possessed with another embryonic organ. Janosik[4] has attempted to trace the earlier history in mammals, and has

[1] "Bau u. Entwick. d. Nebennieren bei Reptilien," Semper's 'Arbeiten,' Bd. v.

[2] "On the Development of the Suprarenal Bodies in Mammalia," 'Quart. Journ. Mic. Sci.,' 1882.

[3] 'Archiv. für Anat. u. Phys.,' 1883.

[4] 'Archiv für Mikr. Anat.,' 1883.

been led to believe that the blastema of Gottschau, Mitsu-
kuri, and others arises as a series of (segmental?) outgrowths
from the peritoneum, in the angle between it and the root of the
mesentery and the peritoneum. As, however, very few figures
are given with this paper it is not easy to form an idea of the
exact nature of the events described.

This state of things led me to believe that it might be
worth while to examine carefully embryos younger than those
used by any previous observers, and so to trace the earlier
history of the cortical blastema. This I have been able to do,
during the summer of the present year, in the chick, in
Lacerta muralis, and in Pristiurus. As my observations
are most complete in the case of Lacerta, I begin with an
account of the development in that type. In order fully to
understand the development of the suprarenal body, it will be
necessary to follow the development of the glomeruli of the
mesonephros, which has been described by Braun (loc. cit.)
After the formation of the segmental vesicles and Wolffian
duct each segmental vesicle gives off from its outer margin a
solid column of cells, which joins the Wolffian duct, and soon
acquires the ဢ shape characteristic of the young segmental
tubes in so many Vertebrates. After this cord of cells has
united with the Wolffian duct, the lumen of the segmental
vesicle extends into it, and it takes on all the characters of
a segmental tubule. After this has happened, one wall of the
persisting segmental vesicle becomes pushed in by a plexus of
blood-vessels, and forms a glomerulus.

But while the wall of the glomerulus is being thus invagi-
nated, a proliferation of the cells composing it occurs at the
side opposite to the point of attachment of the segmental tube,
that is, on the inner margin of the glomerulus.

In fig. 1, I have attempted to represent the condition of things
in one of the anterior glomeruli of an embryo with about
twenty protovertebræ. The section passes nearly through the
centre of the glomerulus, which is seen to be only partially
invaginated; and I may here call attention to the manner in
which, in lizards at least, the invagination seems to take place

before the entrance of the blood-vessels, none of which are
to be seen in the section figured. The epithelium is much
more columnar than at a later stage, and is regularly one cell
thick on the outer side, while on the side undergoing invagina-
tion it is more or less regularly composed of two layers of cells ;
but at every point except one the whole glomerulus is bounded
by cells of a definitely epithelioid character, having no pro-
cesses, and showing no indication whatever of any tendency to
proliferation. At the inner margin, however, the case is
different; here the limiting cells are irregular in shape, and
can in no way be separated, by any sharp line of demarcation,
from the cells forming the Λ-shaped mass (*s. r. b.*), which is
seen to be attached to the inner wall of the glomerulus. This
mass gives rise both to the connecting tubules between testis
and epididymis and to the cortical substance of the suprarenals.
At present it is seen to extend for a short distance dorsal-
wards, between the segmental tubule (*s. t.*) and the vena cava
(*v. c.*), and then to bend rather sharply ventralwards towards
the generative ridge, the anterior end of which (*W. r.*) is seen
in the section. As a contrast to the continuity between the
cell mass in question and the cells bounding the cavity of the
glomerulus I would especially call attention to the distinctness
of the line of demarcation between it and the endothelium of
the vena cava, at the point where the two are in contact—a
distinctness which, persisting, as we shall see it to do, through
all stages of the development of the suprarenal blastema, ren-
ders it extremely difficult to believe that the endothelium is in
a state of proliferation, or that there is any real connection
between it and the suprarenal blastema.

The small blood-vessel (*b. v.*) which is seen in the figure
is also perfectly sharply separated from the adjacent tissues.

The section represented in fig. 2, from an embryo about
4·5 mm. long, with twenty-four protovertebræ, shows a further
advance in the development of the suprarenal blastema and its
associated glomerulus. The section, which passes through the
entrance of a segmental tube into the glomerulus, shows the
completion of the invagination, and the entrance of blood-

vessels (diagrammetrically indicated by shading).   The epithelium of the glomerulus is everywhere, except on its inner side, formed of a single layer of cells, which are much flatter than in the preceding stage, but on the inner side the cells pass, as before, without any definite line of demarcation, into the suprarenal blastema, which is still composed of a compact mass of polygonal cells, without any distinction being visible between the part which is going to form suprarenal body and that which is going to form a seminiferous tubule.   In this section the distinction between the endothelial cells of the various blood-vessels and the tissues surrounding them is even better marked than in the one last described.

The appearances which I have attempted to describe are seen first in the more anterior, then in the hinder glomeruli of all that region of the mesonephros which is coextensive with the generative ridge, and in one or two glomeruli in front of it.

The blastema which I have described grows, in the succeeding stages, in two directions : dorsalwards between the cardinal vein (or vena cava) and the tubules of the mesonephros, and ventralwards into the prominence of the Wolffian ridge.   In such a section as that shown in fig. 3, for example, which is taken from the posterior part of the mesonephros of an embryo of 8 mm., two distinct regions may now be distinguished, a region (*s. r. b.*) dorsal to the point of origin from the glomerulus, the cells composing which will go to form the suprarenal, and a region (*s. str.*) going from the glomerulus ventralwards into the generative ridge, which is the rudiment of the testicular network.   No histological difference can as yet be detected between the one region and the other, the whole blastema being composed of a mass of polygonal cells with rounded nuclei, the characters of which are everywhere identical.

In an embryo of 10 mm. (figs. 4 and 5), a slight distinction between the two parts is for the first time apparent, though the histological characters of adult suprarenal cells are not acquired for some time.   Of the two sections figured, that shown in fig. 4 is taken in front of the Wolffian ridge ; in it,

therefore, the blastema attached to the glomerulus gives rise only to suprarenal tissue. For this figure, I have purposely chosen a section in which the contact between the suprarenal rudiment and endothelium of the vena cava was as close and as extensive as possible, in order to show the distinctness which, in spite of their close apposition, exists between the two structures, and to contrast once more this distinctness of the vena cava endothelium with the irregular way in which the cells of the glomerulus wall are merged in the blastema. This section is also interesting from another point of view. One of the arguments used by Dr. Braun, in order to disprove the existence of any real connection between the rudiment of the testicular network and that of the suprarenal, is that the segmental rudiments of the former structures are well developed before the appearance of any suprarenal tissue at all. Dr. Braun believes that the whole of the outgrowth from each glomerulus becomes converted into a seminiferous tubule. But if this be so, what can be the function of such an outgrowth in front of the testicular region?

In fig. 5 is seen a section through the beginning of the generative ridge : the suprarenal and seminiferous rudiments are still continuous, but the one is a little more deeply stained, and its component cells are a little smaller than the other. As before, the endothelium of the surrounding blood-vessels forms a distinct layer over the blastema, the cells of which are quite sharply defined and clearly recognisable.

The upward growth of the suprarenal rudiment, already well marked in fig. 5, is still better seen in fig. 6, from the middle of the trunk of an embryo of 13 mm.—almost the oldest in which a connection between suprarenal and seminiferous tubules can be seen. In an embryo of 18 mm. (fig. 7), the separation has already taken place, and the suprarenal is cut off by blood-vessels from all adjacent structures, though it remains now, as always before, perfectly distinct from the endothelium of the vessels themselves. This stage is only very slightly younger than the youngest figured by Braun, as fig. 4, Pl. I. of his paper shows ; the

chief difference between his figure and mine being that he has, having overlooked the earlier stages, been led to an erroneous form of opinion as to the mode of origin of the tissue which he figures.   From this point onwards, however, his observations as to the histological differentiation of the cortical substance, and the entrance into it of the medullary ganglion cells are so complete that it is needless to attempt to add anything to his description.

In Pristiurus, as in other forms, the early history of the suprarenals has only been traced from a point at which a mesoblastic rudiment, distinct from all other organs, already existed. This is the stage at which Balfour, in the passage already quoted, begins his account of their development.   I propose, therefore, to trace the history of this blastema in Pristiurus, which is the only Elasmobranch in which I have observed it.

In figs. 9 and 10 are shown two consecutive sections through a Pristiurus embryo 8 mm. in length, at a stage corresponding to Balfour's Stage I—the stage immediately preceding that in which he begins the history.   Both these sections pass through the opening into the body cavity of the same segmental tube, which is seen to give off, just after the narrowing of its funnel-shaped opening into the body cavity, a small process (s. r.) ,which projects towards the root of the mesentery. In fig. 9, which passes through the middle of this process, it is seen to have a very considerable lumen.   In fig. 10 it is cut tangentially, and the lumen is therefore not apparent.

In figs. 11 and 12, from a slightly older embryo, this diverticulum of the segmental tubule is seen to have obtained a considerable size, and to project quite to the middle line over the root of the mesentery.   It is not seen in the figure to be joined by a similar structure from the opposite side, because the section copied was so oblique that the right hand side was intervertebral.   In the next following section, however (fig. 13), the wall of the outgrowth of the other side is cut.

In an embryo of between 9 and 10 mm. the outgrowth has become solid, and lies just over the root of the mesentery, as shown in fig. 14; further, at this stage the outgrowths have

so coalesced with those in front and behind that an interverte-
bral section, such as that shown in fig. 15, still passes through
them.

One feature of the sections of this age, which I do not
understand completely, is the shifting of the position, with
regard to the segmental funnel, of the point of attachment of
the suprarenal outgrowth; while in the preceding stage (see
fig. 12) the outgrowth was external to the primitive ova, open-
ing distinctly into the segmental funnel, it is now attached to
the peritoneal epithelium at the root of the mesentery internal
to the primitive ova. While I am unable to account for
this apparent change of position, I see no reason for doubt-
ing the identity of the structure I have called *s. v.* in figs. 14
and 15 with that similarly named in the preceding figures.

In the next stage, finally, which is a young embryo of
Balfour's Stage IV, we find (fig. 16) the unpaired rod of meso-
blast described by him lying at the root of the mesentery,
but still attached segmentally (see the left hand side of the
figure) to the segmental funnel.

I have unfortunately no stage intermediate between this
and the stage last described, but it seems obvious that the
unpaired blastema existing at this stage must be produced by
the fusion of the paired outgrowths of the earlier stages.

An important point with regard to this blastema in Pris-
tiurus, which has apparently been overlooked by Balfour, is
that it extends throughout the whole length of the mesonephros.

It is well known that in an adult Elasmobranch there are
two sets of suprarenal bodies: one a series of paired, more or
less regularly segmental bodies, attached to the dorsal wall of
the cardinal vein on each side in the mesonephric region, and
the other one unpaired, median body, lying between the two
halves of the metanephros.

Balfour was of opinion that the bodies of the anterior set,
though they show in the adult a division into cortical and
nervous positions as distinct as that which exists in the supra-
renals of higher Vertebrates, were yet derived entirely from
sympathetic ganglia. The presence, in the anterior end of the

body, of a blastema such as I have described seems to throw doubt on the correctness of such a view; though I have unfortunately been unable, owing to want of material, to prove by examination of later stages the share which this blastema takes in the formation of the paired anterior suprarenals.

In the chick, as might perhaps have been expected, from the highly-modified development of the whole kidney, the mode of origin of the suprarenal blastema differs in many important points from that which has been described for the dogfish and for the lizard.

Before the fourth day of incubation there is no trace of any suprarenal rudiment whatever. By about the end of this day, however, certain large cells, the rudiments of the cortical substance, make their appearance in the indifferent mesoblast at the inner side of the mesonephros. The exact mode of origin of these cells I have been unable to determine. At their first appearance they lie, singly or in groups of two or three, in the mesoblast between the aorta and the kidney, being distinguished from the surrounding cells by their rounded, unbranched form, their larger size, and the clearness of their protoplasm. During the end of the fourth day, and the early part of the fifth, they increase in number, either by division or by addition from the surrounding mesoblast, till in an embryo of about the middle of the fifth day of incubation, they form groups of a considerable size, which present in section the appearances seen in fig. 17. The cells seen in this section, though they are more numerous than at the time of their first appearance, have not appreciably changed their relations to the surrounding parts. They are seen to lie surrounded entirely by branched mesoblast cells without any connection, either with the epithelium of the adjacent glomeruli, or with the walls of any blood-vessels. In this isolated condition the suprarenal cells remain during the fifth and sixth days, travelling, however, gradually towards the mesonephric glomeruli, and at the same time increasing in number, and tending to arrange themselves in irregular branched columns, having in section an elliptical outline. During the seventh day they attach them-

selves to the epithelium of the glomeruli, so as to appear as in fig. 18. In this figure the epithelium of the glomerulus is seen to be distinct from the suprarenal for a short distance; but in a part of the section I was unable, after a tolerably careful examination, to convince myself of the existence of any distinct layer of epithelial cells separating the cavity of the glomerulus from the adjacent blastema.

Such a section as that shown in fig. 18 may be seen in almost any glomerulus in the region of the suprarenal during the seventh day. On the eighth day the appearance of the blastema changes. While still retaining its connection with the glomeruli (fig. 19) it has increased considerably in size, and its component cells have acquired most of the histological characters which they present in the adult. The individual cells are large, polygonal, and distinctly marked off one from the other; their protoplasm, which does not stain very readily with carmine or hæmatoxylin, is clear or very finely granular, and their nuclei are clear, oval, or elliptical, with well-defined contours and a number of coarse granules in their interior. The most characteristic feature in the blastema of this age is, however, the definite arrangement of the cells into columns, giving them, more than at any earlier stage, the appearance of the cortical substance of an adult suprarenal.

I have already said that the blastema during the eighth day remains attached to the glomeruli; such appearances as those seen at x in fig. 19, which are very frequent, tempt one strongly to believe that at this time the number of the cells composing it may be added to by proliferation from the glomerulus epithelium; but I have not been able to satisfy myself that this is the case.

From this time the changes in the cortical blastema, so far as I have followed them, do not differ in any important particulars from those described by Braun in Lacerta muralis.

A noticeable feature throughout the whole of the early history of the organ under consideration in the chick, is the very distinct separation between the cortical blastema and the blood-vessels, the original blastema-cells being at a great

distance from any vessel, and the later tissue only approaching one when it has so greatly increased in size as to have pushed all the intervening mesoblast, so to speak, on one side. There is no possibility of believing, in this case at least, that the walls of the blood-vessels have the slightest share in the production of the cortical blastema.

The great difference between the results of the investigations of previous observers and those which have just been described, is sufficiently obvious. If, however, the accuracy of my observations be admitted, we have a much more rational explanation of the phylogeny of the suprarenals than is possible if we adopt the view of Braun, and others;—an explanation which receives support, both from the anatomical relations of the adult organs, and with those of the corresponding organs in Myxinoids and Teleosteans.

In Bdellostoma, I have already[1] attempted to show that the head kidney has become modified so as to form an organ functionally analogous to the suprarenals; while in Teleosteans, a most remarkable series of modifications, affecting every region of the kidney, has been described by Balfour and Emery; a series which seems to me to supply every stage needful to complete our conception of the passage from such a form as Bdellostoma to that of a higher vertebrate. Balfour showed[2] that the head kidney of many adult Teleosteans consisted, not of renal tissue, but of a mass of parenchymatous "lymphatic" material, richly supplied with vessels, whose function, whatever it might be, was certainly not that of a normal kidney. He afterwards found the same kind of modification to exist in the head kidney of the Teleosteoid Ganoids.[3]

Though the observations of Balfour left it highly probable that the "lymphatic" tissue described by him was really a result of the transformation of part of the embryonic kidney,

[1] This Journal, April, 1884.

[2] This Journal, 1882.

[3] "On the Structure and Development of Lepidosteus," 'Phil. Trans.,' 1882.

he did not investigate the details of its development. This was afterwards done by Emery,[1] with the following results :—

In those Teleostei which he has studied, Professor Emery finds that at an early stage the kidney consists entirely of a single pronephric funnel, opening into the pericardium, and connected with the segmental duct, which already opens to the exterior. Behind this funnel, the segmental duct is surrounded by a blastema, derived from the intermediate cell mass, which afterwards arranges itself more or less completely into a series of solid cords, attaching themselves to the duct (see fig. 20). These develop a lumen, and become normal segmental tubules, but it is, if I may be allowed the expression, a matter of chance, how much of the blastema becomes so transformed into kidney tubules, and how much is left as the "lymphatic" tissue of Balfour, this "lymphatic" tissue remaining either in the pronephros only, or in both pro- and mesonephros.

We have here, as it seems to me, an explanation of the reason why the suprarenals, while arising from the pronephros in Myxinoids, are mesonephric in origin in the higher Vertebrates. The same causes which led to the degeneration of the original renal pronephros (causes among which the specialisation of the pericardium, and the development of the air-bladder and lungs may have played a considerable part)—the same causes which led to the establishment of the mesonephros as the chief seat of renal secretion may, and indeed must, have rendered advantageous the suppression of any glandular organ in the pronephric region; and thus, when, in consequence of the change of function of the Wolffian duct more and more of the mesonephros became useless as a kidney, it is easy to understand how some of its component parts underwent in their turn the same change of function as had been undergone by the anterior part of the renal organ at an earlier stage in its evolution, stages in the completion of this process remaining

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[1] 'Atti dell' Academia dei Lincei.,' 1882.

both in the commencing modification of the Teleostean meso-
nephros on the one hand, and on the other in the suprarenal
of Amphibia, with its own " portal " circulation, and its close
connection with the renal tissue.

EXPLANATION OF PLATES XI & XII,

Illustrating Mr. W. F. R. Weldon's Paper "On the Supra-
renal Bodies of Vertebrata."

Complete List of Reference Letters.

Al. Alimentary canal. *Ao.* Aorta. *Bv.* Blood-vessel. *gl.* Glomerulus.
g. ep. Glomerulus epithelium. *pe. ep.* Peritoneal epithelium. *Mes.* Mesentery.
v. c. Vena cava. *s. t.* Segmental tubule. *s. str.* Testicular tubule. *s. r. b.*
Suprarenal blastema. *W. r.* Wolffian ridge.

The figures were in all cases drawn by the aid of a Zeiss's camera lucida.

Fig. 1.—Transverse section through a glomerulus of an embryo of Lacerta
muralis with twenty-one protovertebræ.

Fig. 2.—Similar section through an embryo with twenty-three protover-
tebræ.

Fig. 3.—Similar section through an embryo 8 mm. long.

Fig. 4.—Similar section from an embryo 10 mm. long.

Fig. 5.—Similar section from an embryo of 11 mm.

Fig. 6.—Similar section from an embryo of 13 mm.

Fig. 7.—Similar section from an embryo of 18 mm.

Fig. 8.—Transforming blastema of teleostean kidney, copied from Emery.

Figs. 9 and 10.—Two consecutive sections through an embryo of Pris-
tiurus melanostomus of 8 mm.

Figs. 11—13.—Consecutive sections from an embryo of Pristiurus of $8\frac{1}{2}$ mm.

Figs. 14 and 15.—From an embryo of Pristiurus of 10 mm.

Fig. 16.—From an embryo of Pristiurus slightly older than that figured in
figs. 14 and 15.

Fig. 17.—From a five-day chick.

Fig. 18.—From a seven-day chick.

Fig. 19.—From a nine-day chick.

st.
s.r.h
gl.ep.
b.v.
w.r.
Mes

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st.
g.ep.
pe

b.r.
s.r.h
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w.r.s.

r.c.t

U
gl.ep. pe.ep.

mes
4

Ao

r.c

gl.ep.
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Mes

9

8

b.v.

s.r.b

b.v

v.e.i

w.r.

Mes.

3.

v.e.

a.o

s.r.b

gl.ep.

pe.ep.

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w.r

6.

s.r.b.

s.t

v.e.i.

gl

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r

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W.R

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s.r.b

b.v

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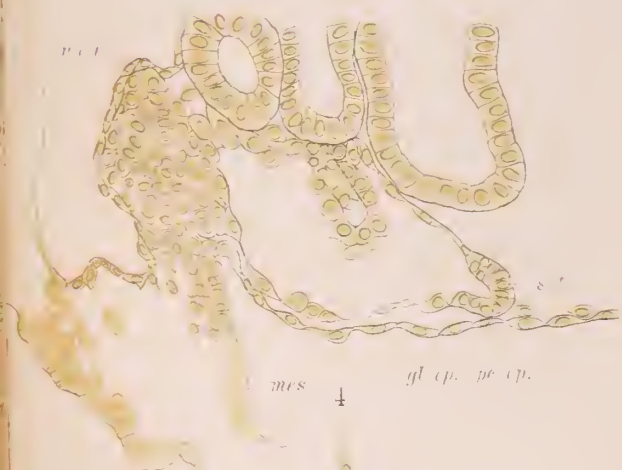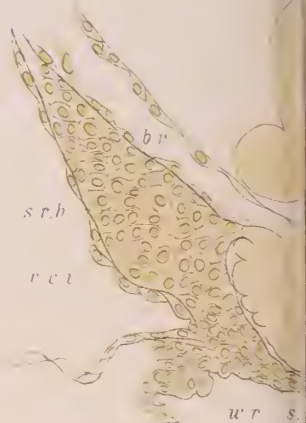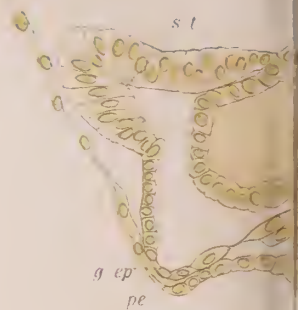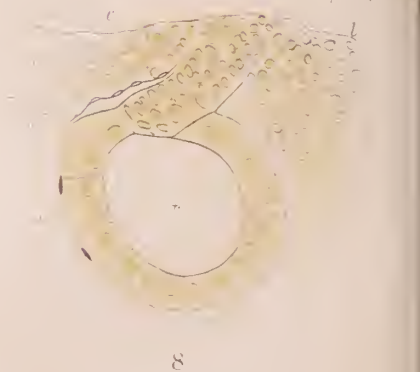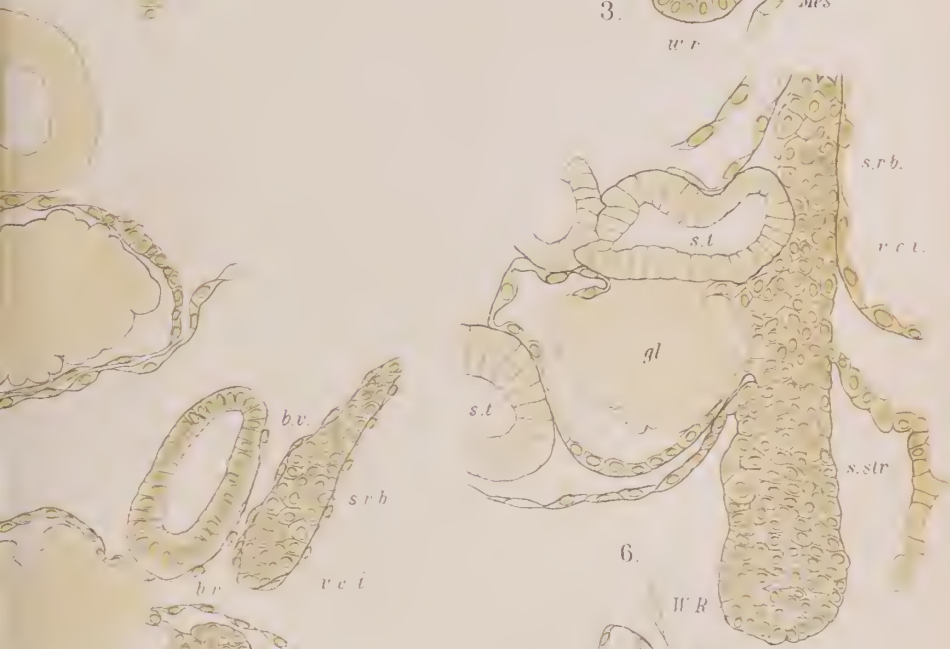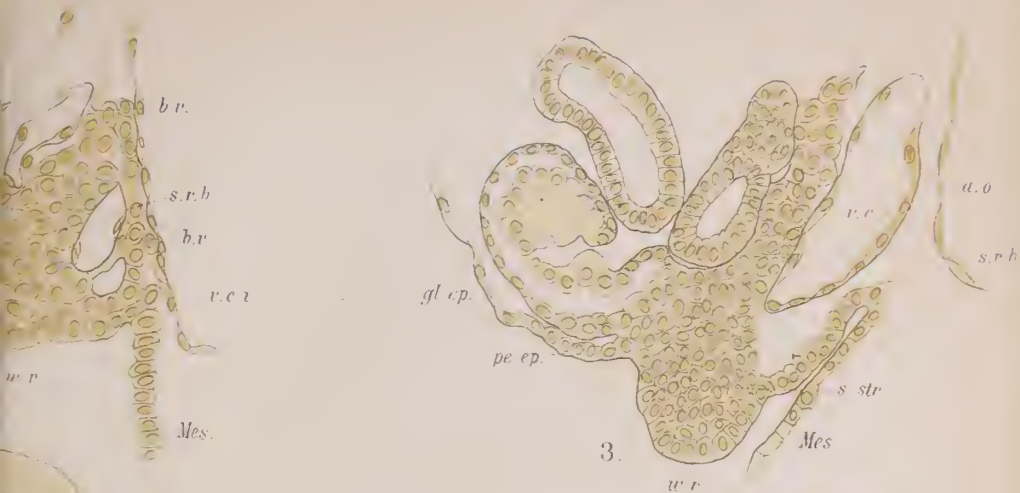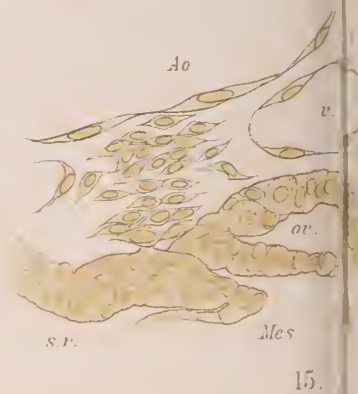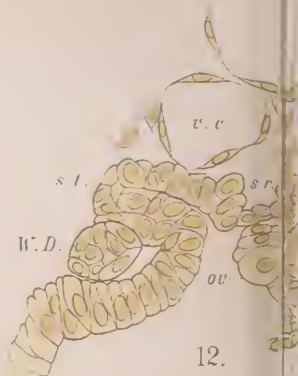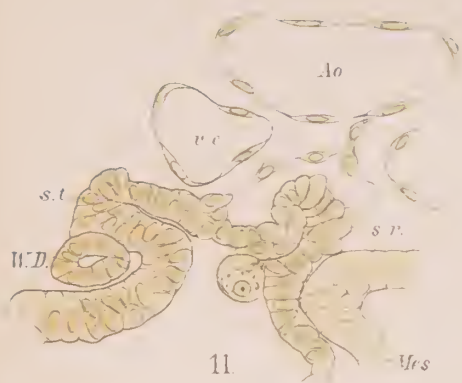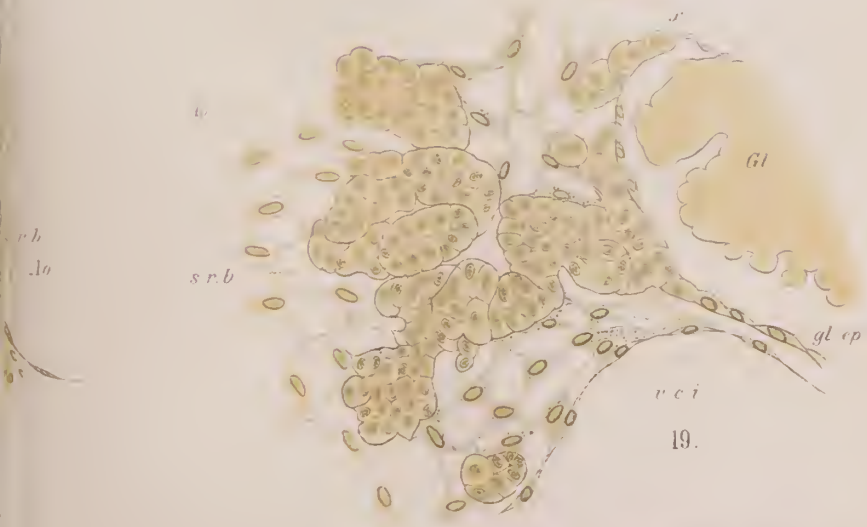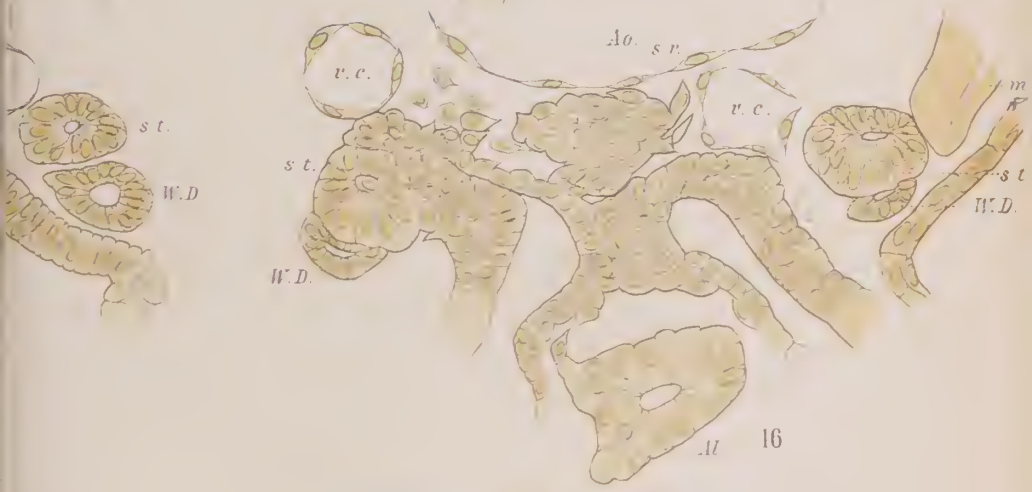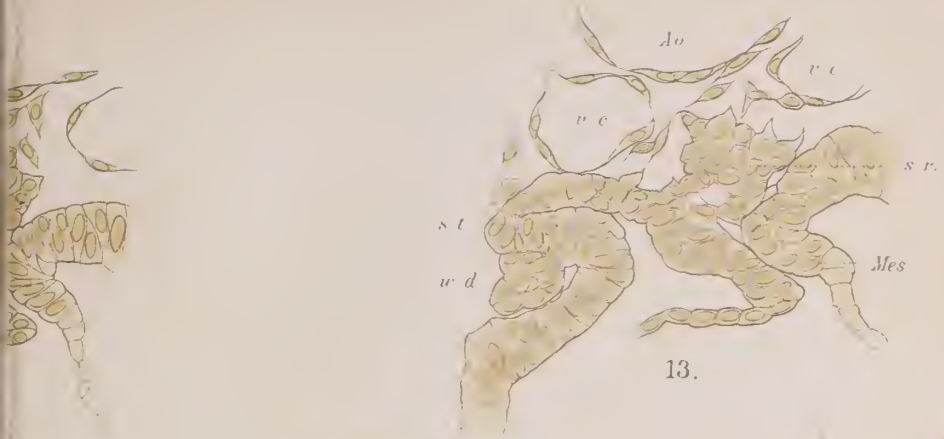
On the Life-History of certain British Heterœcismal Uredines. (The Ranunculi Æcidia and Puccinia Schœleriana.)

By

Charles B. Plowright.

In the following communication, the life-histories of five species of Uredines which during the past three years have been investigated are detailed, together with an enumeration of the experimental cultures performed in connexion therewith, by which it will be seen that the conclusions have not been hurriedly arrived at. It may be thought that many of these cultures are needless repetitions, but I have found myself compelled to differ in certain points with the eminent Continental botanists who have made this subject their special study, and to whom, indeed, we owe all the information we at present have concerning it. It will be seen that these differences are mainly connected with the host plants upon which the various Uredines in question occur. It is hoped that my eminent confrères will recognise the fact that my investigations have not been made in any spirit of captious criticism, but rather with the object of verifying and amplifying the discoveries they have already made. Hence when any statement of theirs has been found to accord with my own results, this particular culture has not been repeated many times. For example, with Uromyces dactylidis twenty-seven experiments were made altogether, but of these only five were confirmatory of Schröter's statement as to its Æcidium occurring upon Ranunculus bulbosus, for the simple reason that Schröter's statement on this point is correct, and it would have been a

mere waste of time to have proved what is already known to be true. The other twenty cultures were made upon other host plants, and were mostly repetitions made over and over again before I felt myself justified in differing from him.

Experiments   1 to 100 were made in 1882.
   „ 101 „ 244 „ „ 1883.
   „ 245 „ 426 „ „ 1884.

The Ranunculi Æcidia.

History of the Subject.—The various members of the Ranunculus family are peculiarly liable, as Schröter[1] has pointed out, to be affected with the æcidiospores of various Uredines. In fact in this country no less than eleven species have Æcidia occurring more or less frequently upon them, whereas only four species have either teleutospores or uredospores affecting them. The æcidial host plants belonging to the Ranunculaceæ in this country are Clematis vitalba, L.; Thalictrum alpinum, L.; flavum, L.; Anemone nemorosa, L.; ranunculoides, L.; Ranunculus acris, L.; repens, L.; bulbosus, L.; ficaria, L.; Caltha palustris, L., and Aquilegia vulgaris, L.; while teleutospores and uredospores only occur on Thalictrum flavum, L.; Anemone nemorosa, L.; Ranunculus ficaria, L.; and Caltha palustris, L.

The author above quoted has shown that many of these Æcidia are heterœcismal. He pointed out that those writers who, like Fuckel[2] and Cooke,[3] have affiliated the very common Æcidium upon R. ficaria with the Uromyces, which also occurs upon this plant, were wrong in so doing; that these two fungi are distinct species, having separate and altogether unlike life-histories, and that their occurrence upon the same host plant is a mere accidental circumstance. He further showed that the Æcidium in question is really connected with a Uromyces which

[1] Schröter, 'Cohn's Beiträge zur Biologie der Pflanzen,' vol. iii, Heft 1, p. 59.

[2] Fuckel, 'Symbol. Mycol.,' p. 64.

[3] Cooke, 'Uromyces in Grevillea,' vol. vii, p. 136.

affects the various Poæ. This he did by a series of artificial cultures. He was led to try these experiments from his previously obtained knowledge of the life-history of Uromyces dactylidis, which he found had its uredo and teleutospores upon Dactylis glomerata while its æcidiospores occur upon Ranunculus bulbosus. The results obtained by Schröter were (1) that Uromyces dactylidis, Otth, is produced from a Uredo upon Dactylis possessing capitate paraphyses having its Æcidia upon Ranunculus bulbosus and repens. (2) That the Uredo without paraphyses and its Uromyces upon Poa nemoralis are connected with the Æcidium upon R. ficaria. Winter, in his latest work,[1] following Schröter, thus gives the relationships of these species.

<div align="center">Uromyces dactylidis, Otth.</div>

Æcidiospores on.	Teleutospores on.
Ranunculus acris, L.	Arrhenatherum elatius, M. and K.
,, polyanthemos, L.	Poa nemoralis, L.
,, repens, L.	Dactylis glomerata, L.
,, bulbosus, L.	Festuca elatior, L.

<div align="center">Uromyces poa Rabh.</div>

Æcidiospores on.	Teleutospores on.
Ranunculus ficaria, L.	Poa nemoralis, L.
	,, pratensis, L.

Cornu[2] has, however, still more recently shown that Ranunculus repens is the host plant of the Æcidium of Puccinia arundinacea, D. C. He further considers that P. graminis, Pers., occurs upon Phragmitis communis not unfrequently, when it is characterised amongst other things by forming long black lines on the stem.

Rostrup[3] regards the Æcidium on Ranunculus repens as being due to Uromyces poæ, Rbh.

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[1] Winter, 'Rabenhorst's Kryptogamen Flora,' vol. i, p. 162.

[2] Cornu, 'Comptes Rendus,' 26 Juin, 1882.

[3] I regret having mislaid the communication which Mr. Rostrup sent me. It was a list of Danish fungi, in which the æcidiospores of Uromyces poæ were given as occurring upon Ranunculus ficaria and repens.

Personal Investigations.—The above being the state of our information concerning the affinities of the Ranunculi Æcidia, the following series of experimental cultures have oeen made in the hope of definitely clearing up the matter. These were commenced in 1882 and continued through 1883 and 84.

Contradictory as the above views appear to be in many points, yet it will be seen that they are none the less in the main correct.

In order to render this communication the more lucid at the expense of increasing its length to some extent, each species will be treated separately, and have appended to it a tabular statement of the cultures made respecting it.

Uromyces poæ, Rbh.—This Uromyces occurs in England very abundantly upon Poa trivialis, L., and P. pratensis, L. I have not met with it upon any other species of Poa nor upon any other grass. Of course it may occur upon other grasses, but I have never found it, nor have I been able to produce it upon any other. As Schröter has shown, its Æcidium is very abundant from February to May upon Ranunculus ficaria. But not only is R. ficaria its host, but also R. repens. This latter fact was suggested to me before I knew of Rostrup's views from the profusion with which the above-named Poæ were attacked by the Uromyces in localities in which R. ficaria did not occur, and in which R. repens did. A series of seven experiments were made, however, by placing the spores of Æcidium Ranunculi repentis upon Dactylis glomerata (Expts. 29, 30, 119, 122, 131, 153, 154) before Schröter and Winter's statement that it is connected with Uromyces dactylidis was definitely rejected. The material employed in these experiments was not collected from a single locality ; on the contrary, specimens were procured from several places near King's Lynn, and even from Shrewsbury, kindly sent to me by Mr. W. Phillips, F.L.S. Moreover, the Æcidium upon R. repens gave rise to no Uredo upon P. nemoralis (143) nor upon P. annua (155). Conversely the germinating spores of Uromyces dactylidis in five cultures upon

R. repens gave no result (118, 249, 254, 270, 290); we may therefore conclude that Uromyces dactylidis is not connected with the Æcidium upon R. repens.

On the other hand, the Æcidium on R. repens was found to produce the Uredo and Uromyces upon Poa trivialis (190, 322, 333). But here another difficulty confronted me, inasmuch as no less than in seven other cultures with the spores of the Æcidium on R. repens applied to P. trivialis and P. pratensis did I fail to obtain any result (120, 146, 147, 155, 191, 336, 370). It is all very well to say that one positive result is of more value than an indefinite number of negative results, but succeeding only in three out of ten cultures requires some satisfactory explanation. Those who have performed cultures with the Uredines know well enough how easy it is to fail from a variety of causes. I have failed more than once in infected wheat plants with Uredo linearis in which no question of specific identity is concerned. It is furthermore very easy to fail with cultures in which æcidiospores are employed as the infecting material, because, in the first place, the æcidial cup is full of spores, but only the few mature ones at its orifice will germinate at all; and in the second place, because even these ripe spores very rapidly lose their germinative power. Still these facts were well enough known to me, at any rate in my later cultures when I had also gained some knowledge of the minutiæ required for successfully manipulating with these bodies. As will be seen later, the explanation simply is that upon Ranunculus repens another Æcidium occurs (that of P. Magnusiana) which so closely resembles it in appearance, and in the form, size, and colour of its spores, that I am quite unable to tell the one from the other. I do not say that this cannot be done by others more skilled in the differentiation of uredine spore forms, but up to the present I have been unable to do so.

The Æcidium on R. ficaria gave no result on P. nemoralis (133, 296), nor upon Dactylis glomerata (297), but upon P. trivialis (295) it gave rise to the Uredo of Uromyces Poæ.

No. of Expt.	Infecting Material.		Plant Infected.		Date of Infection.	1st Result.
190.	Æcidium Ranunculi repentis		Poa trivialis		19 June.	10 July.
322.	,,	,,	,,	,,	25 Apr.	14 May.
333.	,,	,,	,,	,,	9 May	20 May.
295.	,,	Ranunculi ficariæ	,,	,,	9 Apr.	10 May.
29.	,,	Ranunculi repentis	Dactylis glome-rata		13 May	—
30.	,,	,,	,,	,,	13 May	—
119.	,,	,,	,,	,,	28 Apr.	—
122.	,,	,,	,,	,,	28 Apr.	—
131.	,,	,,	,,	,,	2 May	—
153.	,,	,,	,,	,,	31 May	—
154.	,,	,,	,,	,,	31 May	—
143.	,,	,,	Poa nemoralis		21 May	—
155.	,,	,,	,, trivialis and annua		1 June	—
118.	Uromyces dactylidis		Ranunculus re-pens		28 Apr.	—
249.	,,	,,	,,	,,	2 Feb.	—
254.	,,	,,	,,	,,	8 Feb.	—
270.	,,	,,	,,	,,	5 Mar.	—
290.	,,	,,	,,	,,	7 Apr.	—
120.	Æcidium Ranunculi repentis		Poa pratensis		28 Apr.	—
146.	,,	,,	,,	,,	21 May	—
147.	,,	,,	,,	,,	26 May	—
191.	,,	,,	,, trivialis		16 June	—
336.	,,	,,	,,	,,	9 May	—
370.	,,	,,	,,	,,	21 May	—
133.	,,	Ranunculi ficariæ	,, nemoralis		2 May	—
296.	,,	,,	,,	,,	9 Apr.	—
297.	,,	,,	Dactylis glome-rata		9 Apr.	—

Puccinia Magnusiana.—There are certainly two Pucciniæ which occur in this country upon one common reed (Phragmitis communis, Trin.), the one characterised by its teleutospores being born upon very long pedicels and its brown uredo-

spores not being mixed with paraphyses. This is P. phragmitis, Schum = (P. arundinacea, D. C.). The other, P. Magnusiana, Körn, has its teleutospores with much shorter pedicels, and they often form long black lines running down the sheaths and stems of the affected plant. Its uredospores, moreover, are deep orange in colour, and always, as far as I know, mixed with paraphyses. This species has in this country hitherto generally been regarded as a variety of Puccinia graminis, Pers.

In a previous paper[1] I have shown that P. phragmitis has its æcidiospores upon Rumex conglomeratus, crispus, obtusifolius, hydrolapathum, and Rheum officinale (Expts. 140, 178, 179, 180, 181, 182, 183, 334), and conversely that the æcidiospores of Æc. rumicis thus produced, when placed upon Phragmitis, gave rise to the brown Uredo of P. phragmitis without paraphyses (148, 166, 172, 188, 189, 208, 209). This being the case, it occurred to me, after reading Cornu's[2] communication that it was just possible he might have confounded these two Pucciniæ, and that P. Magnusiana might have its Æcidium upon Ranunculus repens. This possibility was to me the more probable, because I had in 1882 fallen into a similar error, and I had then operated in the same manner as M. Cornu appears to have done, namely, by placing leaves and stems of the Phragmites upon the plant to be infected. Since 1882 all my cultures with these Pucciniæ have been made by germinating the teleutospores in a watch-glass, having previously separated them from the reed, and examining them microscopically to insure against error from the commingling of the spores of the two species. Now, it happened that after reading M. Cornu's paper I found, in the autumn of 1883, a long, straight ditch full of reeds upon which at both ends, for about twenty yards, the Phragmites were completely blackened by P. Magnusiana. This ditch was about a quarter of a mile in length, and the reeds which

[1] Plowright, "On the Life-History of the Dock Æcidium," 'Proc. Royal Soc.,' No. 228, 1883, pp. 47—49.
[2] Cornu, 'Comptes Rendus,' 26 June, 1882.

grew in the intervening part were quite free from the Puccinia. Neither did I observe anywhere in it a single pustule of P. phragmitis. In the spring of 1884 I from time to time visited this ditch and carefully examined the Rumices and Ranunculi growing on its banks, for I felt certain, from the localised profusion with which P. Magnusiana occurred, that I should meet with its Æcidium at both ends, but not in the central part. This surmise was found to be correct, and to confirm the conclusion I had already, from a series of experimental cultures, arrived at. At both ends of the ditch I found the Rumices free from Æcidia, but the plants of Ranunculus repens abundantly affected with Æcidia, while in the middle neither one or other of these plants had any Æcidium upon them. Germinating spores of P. Magnusiana were placed upon Ranunculus repens with the uniform result of giving rise to the Æcidium (315, 335, 358). It is obvious that if Ranunculus repens is the host plant to two specifically distinct Æcidia, the possibility of other Ranunculi also being hosts of one or other of them must be considered. P. Magnusiana was, therefore, applied to R. acris (360), R. ficaria (361), R. auricomus (359), but without any result. When, however, R. bulbosus was infected it always developed the Æcidium (393, 394, 395, 396, 397), hence P. Magnusiana has its Æcidium upon both R. repens and R. bulbosus. Conversely, the æcidiospores of P. Magnusiana which had been artificially produced upon R. repens (369) and R. bulbosus (422), were placed on Phragmitis, where in due time they gave rise to the orange Uredo with paraphyses. To make more sure, a part of the same spores from R. repens, which, when placed on Phragmitis (369) gave origin to the Uredo of P. Magnusiana, were applied to Poa trivialis (370), but they gave rise to no Uredo; and in like manner a part of the spores from the Æcidium R. bulbosus (423) were placed upon Dactylis glomerata, but they gave rise to no Uredo. The two Æcidia on R. repens were most carefully examined side by side, but no difference could be detected by me. As these two Æcidia occur in a state of nature, however,

that of Uromyces poæ occurs rather earlier in the year than
that of P. Magnusiana.

Puccinia phragmitis gave rise to no Æcidium on Ranun-
culus repens (137, 138), nor on R. ficaria (142), although
the teleutospores were in active germination when employed.

But the subject is not even yet fully exhausted. The emi-
nent Danish botanist, Mr. P. Nielsen, in 1879,[1] found that the
Æcidium on Rumex acetosa was developed from P. phrag-
mitis, and Winter [2] gives his adherence to this view. Now,
Mr. Nielsen is a most careful and expert experimenter with
the Uredines. I therefore performed the following experi-
ment. A quantity of P. phragmitis was placed in water in
a watch-glass, when it was found, by microscopical examination,
that the spores were in active germination ; one half was
placed on a plant of Rumex acetosa (347), and the other
half upon one of R. obtusifolius (346). The infected plants
were both treated alike, but while in nine days the R. obtusi-
folius became affected with Æcidium rumicus, the R.
acetosa remains to this date (Oct. 31st) free. This method
of experimenting in duplicate is a very valuable one, and I
have frequently employed it inasmuch as it lessens the possi-
bility of error. P. phragmitis was also applied to R. acetosa
(177), but without result, as was also the case when P. Mag-
nusiana was employed (139, 179, 206, 323). Of course,
these are only negative results, but it is at least remarkable
that I should have had no difficulty in producing Æc. rumicis
on the other Rumices, but always have failed with R. acetosa.
It happens, however, that upon Phragmitis Professor Oude-
mans[3] has recorded the occurrence in Holland of P. straminis;
and in the early part of the year 1882 Mr. Bloome sent me,
from near Worthing, a Puccinia on reed, which I regarded at

[1] Rostrup, 'Heteroeciske Uredineer,' p. 10 ; 'Observations nouvelles sur
les Urédinées à generations alternantes,' p. 3 ; 'Aftryk. af Oversigt over
d. K. D. Vidensk. Selsk.,' Fordhandl, 1884.

[2] Winter, 'Rabenhorst's Kryptogamen Flora,' 1881, p. 222.

[3] Oudemans, 'Bijdrage over niew ontdekte Champignons voor de Flora van
Nederland,' 1871, p. 22.

that time as being either P. straminis or P. sessilis, but, unfortunately, the specimen was not preserved by me. This being the case, it is possible that the Puccinia which Oudemans and myself have seen on reed may be a third species connected with the Æcidium on Rumex acetosa; and as it is of such an inconspicuous appearance, may have accidentally crept into Mr. Nielsen's cultures. This, of course, is a mere conjecture upon my part, which can only be confirmed by direct observations. Lastly, with regard to the belief that P. Magnusiana is only a form of P. graminis occurring upon reed, a duplicated experiment was performed in which P. Magnusiana, taken from the black lines on the stem, was placed upon Berberis vulgaris (362) and Ranunculus repens (358); on the former it gave no result, on the latter in ten days its Æcidium.

No. of Expt.	Infecting Material.		Plant Infected.		Date of Infection.	1st Result.
315.	Puccinia Magnusiana		Ranunculus re- pens		24 Apr.	10 May.
335.	,,	,,	,,	,,	9 May	2 June.
358.	,,	,,	,,	,,	18 May	28 May.
393.	,,	,,	,,	bulbosus	7 June	15 July.
394.	,,	,,	,,	,,	7 June	15 July.
395.	,,	,,	,,	,,	7 June	15 July.
396.	,,	,,	,,	,,	7 June	15 July.
397.	,,	,,	,,	,,	7 June	15 July.
34.	,,	,,	Rumex conglom- eratus		18 May	—
70.	,,	,,	,,	,,	15 June	—
81.	,,	,,	,,	,,	15 June	—
167.	,,	,,	,,	,,	5 June	—
72.	,,	,,	,,	obtusifolius	15 June	—
169.	,,	,,	,,	,,	5 June	—
168.	,,	,,	,,	crispus	5 June	—
171.	,,	,,	,,	hydrola- pathum	7 June	—
205.	,,	,,	,,	,,	28 June	—
184.	,,	,,	Rheum officinale		12 June	—
187.	,,	,,	,,	,,	13 June	—

No. of Expt.	Infecting Material.	Plant Infected.	Date of Infection.	1st Result.
{ 422.*	Æcidium Ranunculi bulbosi	Phragmitis communis	1 July	20 July.
423.*	,, ,,	Dactylis glomerata	1 July	—
{ 369.*	,, Ranunculi repentis	Phragmitis communis	21 May	9 June.
370*	,, ,,	Poa trivialis	21 May	—

* From Puccinia Magnusiana.

140.	Puccinia phragmitis	Rumex crispus	16 May	27 May.
178.	,, ,,	,, conglomeratus	8 June	22 June.
179.	,, ,,	,, obtusifolius	8 June	19 June.
180.	,, ,,	,, ,,	8 June	19 June.
181.	,, ,,	,, hydrolapathum	8 June	19 June
334.	,, ,,	,, ,,	9 May	1 June.
182.	,, ,,	Rheum officinale	8 June	19 June.
183.	,, ,,	,, ,,	8 June	19 June.
148.	Æcidium Rumicis	Phragmitis communis	27 May	4 June.
166.	,, ,,	,, ,,	3 June	12 June.
172.	,, ,,	,, ,,	7 June	—
188.	,, ,,	,, ,,	16 June	10 July.
189.	,, ,,	,, ,,	16 June	10 July.
208.	,, ,,	,, ,,	2 July	20 July.
209.	,, ,,	,, ,,	2 July	30 July.
137.	Puccinia phragmitis	Ranunculus repens	18 May	—
138.	,, ,,	,, ,,	18 May	—
142.	,, ,,	,, ficaria	20 May	—
{ 346.	,, ,,	Rumex obtusifolius	16 May	25 May.
347.	,, ,,	,, acetosa	16 May	—
177.	,, ,,	,, ,,	8 June	—
139.	,, Magnusiana	,, ,,	17 May	—
179.	,, ,,	,, ,,	5 June	—
206.	,, ,,	,, ,,	28 June	—
323.	,, ,,	,, ,,	26 April	—
360.	,, ,,	Ranunculus acris	18 May	—
361.	,, ,,	,, ficaria	18 May	—
359.	,, ,,	,, auricomus	18 May	—
362.	,, ,,	Berberis vulgaris	18 May	—

Uromyces dactylidis, Otth.—In 1861 Otth[1] described this Uromyces as occurring upon Dactylis glomerata accompanied by its uredospores. He describes the latter, but makes no allusion to their being associated with paraphyses, while upon the contrary, in another part of the same communication,[2] he describes an Epitea on Dactylis with " colourless, clavate, rather short epiphyses." In 1873 Schröter discovered that U. dactylidis has its æcidiospores upon Ranunculus bulbosus. In 1878 he states[3] that the æcidiospores occur not only on R. bulbosus, but also upon R. repens, L.; R. acris, L.; R. polyanthemos, L. Winter,[4] more recently, while giving the same æcidial host plants, states that the Uromyces occurs not only upon Dactylis glomerata, but also upon Poa nemoralis, L.; Festuca elatior, L., and Avena elatior, L.

Both these last-named authors consider the uredospores of the Dactylidis to be characterised by the possession of capitate paraphyses. Near King's Lynn Uromyces dactylidis occurs in one locality sufficiently near for me to obtain material for experiment and also to watch its growth as it occurs naturally.

Seven attempts made to produce the Uromyces upon Dactylis from the spores of the Æcidium in R. repens uniformly failed (29, 30, 119, 122, 131, 153, 154), and conversely, five attempts to produce the Æcidia upon R. repens from the teleutospores of the Uromyces also failed (118, 244, 254, 270, 290). The æcidiospores applied to Poa nemoralis (143) also produced no effect. But when the germinating teleutospores of this Uromyces were placed upon R. bulbosus they invariably gave rise to the Æcidia (248, 269). In order to see whether this fungus had its Æcidia upon any other of the commoner species of Ranunculus duplicated experiments were performed on R. acris (250, 271), R. ficaria (251, 255, 272), and

[1] Otth, in 'Mittheilungen der Naturf. Gesselschaft,' Berne, 1861, p. 85.
[2] Otth, loc. cit., p. 81.
[3] Schröter, in 'Cohn's Beiträge,' Band iii, pp. 58, 59.
[4] Winter, 'Rabenhorst's Kryptogamen Flora,' 1881, p. 162.

R. auricomus (252, 273), but with no result.   Hence it appears that Uromyces dactylidis has its æcidiospores upon R. bulbosus only, and not upon R. repens or acris.

Spores from the Æcidia on R. bulbosus were placed upon Dactylis glomerata (279, 289, 298), where they in all cases gave rise to a Uredo followed by the Uromyces dactylidis. Æcidiospores of this Æcidium placed upon Poa pratensis (299) and P. amma (300) gave no result.   These last two cultures were duplicated with expt. 298.

In no case, however, in which the Uredo was produced upon Dactylis could I find the least trace of any paraphyses, nor could any be found upon the Dactylis uredo, as it occurs naturally here.   The question, therefore, presents itself, Have Dr. Schröter and myself the same fungus in view?   It is very unlikely that there should be two Uromyces upon Dactylis both having their Æcidia upon R. repens.   Personally, I rather incline to the belief, and it is only a belief which subse-quent observation must confirm or disprove, that these paraphyses are found in certain conditions of the Uredo and not in others; that in other words, their value as a specific character is not of vital importance.   But what these condi-tions are which favour the development of paraphyses, I am unable, at present at any rate, to say.   Just as I was unable to discover any difference externally between the two Æcidia upon R. repens, so am I unable to point out any anatomical differences between the two Æcidia on R. bulbosus, namely, that of Uromyces dactylidis and Puccinia Magnusiana.   Physiologically, however, they are distinct enough.

No. of Expt.	Infected Material.		Plant Infected.		Date of Infection.	1st Result.
248.	Uromyces dactylidis		Ranunculus bulbosus		2 Feb.	6 Mar.
269.	,,	,,	,,	,,	5 Mar.	26 Mar.
279.	Æcidium Ranunculi bulbosi		Dactylis glomerata		17 Mar.	25 Apr.
289.	,,	,,	,,	,,	7 Apr.	20 Apr.
298.	,,	,,	,,	,,	9 Apr.	20 May.
29.	,,	Ranunculi repentis	,,	,,	13 May	—
30.	,,	,,	,,	,,	13 May	—
119.	,,	,,	,,	,,	28 Apr.	—
122.	,,	,,	,,	,,	28 Apr.	—
131.	,,	,,	,,	,,	2 May	—
153.	,,	,,	,,	,,	31 May	—
154.	,,	,,	,,	,,	31 May	—
118.	Uromyces dactylidis		Ranunculus repens		28 Apr.	—
249.	,,	,,	,,	,,	2 Feb.	—
254.	,,	,,	,,	,,	8 Feb.	—
270.	.,	,,	,,	,,	5 Mar.	—
290.	,,	,,	,,	,,	7 Apr.	—
143.	Æcidium Ranunculi repentis		Poa nemoralis		2 May	—
250.	Uromyces dactylidis		Ranunculus acris		2 Feb.	—
271.	,,	,,	,,	,,	5 Mar.	—
251.	,,	,,	,,	ficaria	2 Feb.	—
255.	,,	,,	,,	,,	8 Feb.	—
272.	,,	,,	,,	,,	5 Mar.	—
252.	,,	,,	,,	auricomus	2 Feb.	—
273.	,,	,,	,,	,,	5 Mar.	—
299.	Æcidium Ranunculi bulbosi		Poa pratensis		9 Apr.	—
300.	,,	,,	,, trivialis		9 Apr.	—

Puccinia perplexans, n. sp.—In the spring of this year
I found in two places near King's Lynn upon Alopecurus
pratensis, L., Avena elatior, L., and upon some blades of
grass which I believe belonged to one of the Poæ, the exact
species of which I was unable to determine, an abundant golden
yellow Uredo, the spores of which were freely mixed with well-
developed capitate paraphyses. Naturally the conclusion was
arrived at that here was the Uredo with paraphyses, which
Winter and Schröter have associated Uromyces dactylidis.
The localities were from time to time revisited, but instead of

finding the teleutospores of this paraphysed Uredo to be a
Uromyces they were found to be those of a Puccinia.   Further
search was rewarded by gathering the last year's teleutospores
sparingly upon the Poa, but abundantly upon the other two
grasses.   These Puccinia spores germinated well, and were used
in the following experiments.   In both localities above referred
to numerous plants of Ranunculus acris were found in
close proximity to the grasses affected with the Æcidium.
The germinating teleutospores from the last year's Puccinia
from the Poa (?) Avena elatior and Alopecurus were applied
to R. acris (373, 381, 382, 383, 388, 389), and in every
instance the Æcidium was produced.   Here, then, was quite
an unexpected discovery, that instead of the Æcidium on
R. acris being connected with a Uromyces at all, it was
connected with a Puccinia.   Conversely the æcidiospores in
question were placed upon Alopecurus (401, 402, 404) and
Avena elatior (405), with the result of giving rise to a Uredo,
followed in due course by the Puccinia.   This Uredo, however,
strange to say, was never once accompanied by any paraphyses
at all.   This puzzled me very much, for the paraphysed Uredo
as it occurred naturally was always accompanied by the
Puccinia, but by culture the result was invariably as above
stated.   The possibility of this Puccinia giving rise to a para-
physed Uredo upon some other graminacious host suggested
itself.   The æcidiospores from Ranunculus acris were,
therefore, applied to Poa trivialis (364), P. nemoralis
(367), P. pratensis (365, 366), P. compressa (?) (378),
Dactylis glomerata (379), Lolium perenne (377, 403),
upon all of which paraphysed uredospores are known to occur,
but in every case without any result.

Ranunculus acris was infected with the germinating
teleutospores of Uromyces dactylidis (250, 271) but with
no success, as was also the case when P. Magnusiana (360) was
employed.   Puccinia perplexans bears a strong anatomical
resemblance to P. rubigo-vera, the most obvious difference
being that the last-named species has its teleutospores sur-
rounded by a bed of dark brown paraphyses.   In point of fact

I have previously mistaken these two Puccinia the one for the other. P. perplexans upon Lycopsis arvensis (311, 312, 326), Symphytum officinale (314, 329, 330), Borago officinale (327), and Pulmonaria officinale (328) gave no result. The same was the case when Ribes grossularia (371) and Lonicera periclymenum (313, 317) were infected with it.

Puccinia perplexans may be thus described—

I. Æcidiospores = Æcidium Ranunculi acridis.— Spores, 20 to 25 μ. in diameter, rather more orange in colour than those of the other Ranunculi Æcidia, otherwise not distinguishable.

II. Uredospores.—Sori rubrotund elliptical, but mostly linear. On both surfaces of the leaves, especially on the upper, scattered but sometimes confluent, soon naked golden yellow. Spores, globose, oval or ovate, orange, finely echinulate 20 to 25 μ. wide by 30 to 35 μ. long. With or without capitate paraphyses.

III. Teleutospores.—Sori small, almost black, punctiform, linear, or elliptico-elongate, covered by the epidermis, often clustered and confluent. Spores very irregular in form and size. Clavate, oblong, or subfusiform on very short pedicels, apex sometimes thickened, sometimes not ; upper cell rounded, truncate, or attenuated, often obliquely ; lower cell generally somewhat cuneiform, central constriction slight or absent. Epispore pale clear brown, often apparently coarsely granular, 40 to 60 μ. long by 10 to 12 μ. wide.

I. On Ranunculus acris. II. and III. On Alopecurus pratensis, Avena elatior and Poa sp. ? Near King's Lynn. May and June, 1884.

No. of Expt.	Infecting Material.		Plant Infected.		Date of Infection.	1st Result.
373.	Puccinia perplexans		Ranunculus acris		23 May	9 June.
381.	,,	,,	,,	,,	29 May	9 June.
382.	,,	,,	,,	,,	29 May	9 June.
383.	,,	,,	,,	,,	29 May	12 June.
388.	,,	,,	,,	,,	31 May	9 June.
389.	,,	,,	,,	,,	31 May	9 June.
401.	Æcidium Ranunculi acridis		Alopecurus pratensum		13 June	27 June.
402.	,,	,,	,,	,,	13 June	28 June.
404.	,,	,,	,,	,,	16 June	30 June.
405.	,,	,,	Abena elatior		1 July	20 July.
364.	,,	,,	Poa trivialis		19 May	—
367.	,,	,,	,, nemoralis		19 May	—
365.	,,	,,	,, pratensis		19 May	—
366.	,,	,,	,, ,,		19 May	—
378.	,,	,,	,, compressa (?)		28 May	—
379.	,,	,,	Dactylis glomerata		28 May	—
377.	,,	,,	Lolium perenne		28 May	—
403.	,,	,,	,, ,,		13 June	—
250.	Uromyces dactylis		Ranunculus acris		2 Feb.	—
271.	,,	,,	,, ,,		5 Mar.	—
360.	Puccinia Magnusiana		,, ,,		18 May	—
311.	,,	perplexans	Lycopsis arvensis		23 Apr.	—
312.	,,	,,	,, ,,		23 Apr.	—
326.	,,	,,	,, ,,		5 May	—
314.	,,	,,	Symphytum officinale		23 Apr.	—
329.	,,	,,	,, ,,		5 May	—
330.	,,	,,	,, ,,		5 May	—
327.	,,	,,	Borrago officinalis		5 May	—
328.	,,	,,	Pulmonaria officinalis		5 May	—
371.	,,	,,	Ribes grossularia		23 May	—
313.	,,	,,	Lonicera periclymenum		23 Apr.	—
372.	,,	,,	,, ,,		23 May	—

Puccinia Schœleriana, n. sp.

For many years past I have found on North Wootton Heath, near King's Lynn, an Æcidium on Senecio Jacobæa,

L. This Æcidium is far from common in Great Britain, but in the spring of 1882 I met with it again at Skegness, Lincolnshire. It has been hitherto regarded either as a spore form of Puccinia compositarum, Mart., or of P. Senecionis, Desm. In neither of the above localities was the Æcidium accompanied by any Uredo or teleutospores upon the same host plant. The North Wootton Station has been examined with this point in view repeatedly, and at all seasons of the year. Growing in company with the Senecio, both in Norfolk and Lincolnshire, was, amongst other plants, Carex arenaria, L. In 1882 I noticed upon this Carex a Puccinia occurred on those plants which grew in the vicinity of the Æcidium-affected Seneciones, but not elsewhere. A series of experimental cultures were consequently undertaken, 1883-4, with the object of elucidation of the life-history of the Puccinia in question. There are, as is already known, several well-marked species of Puccinia which occur upon various carices, of these P. carices, Schum.; P. limosæ, Mag.; P. sylvaticæ, Schröt., and P. dioica Mag., have had their life-histories worked out; whereas P. microsora, Körn; P. caricicola, Fcke.; and P. vulpina, Schröt. have not. The only species with which the Puccinia on P. arenaria can be compared is P. dioica, Mag. I therefore sent specimens of my plant to Dr. Magnus, who at once pointed out the difference between the teleutospores of the two Puccinia. In P. dioica the summits of the teleutospores are not only much more thickened, but also generally prolonged upwards into a conical point. The uredospores of P. dioica, too, are described by Dr. Magnus[1] as being similar to those of P. caricis, Schum. Further, Rostrup[2] has, to say the least, pointed out the strong presumptive evidence that exists that Puccinia dioica has its æcidiospores upon Carduus palustris, L.; arvensis, L., and lanceolatus, L. It occurred to me that as Puccinia caricis, Schum., is our commonest carex infesting Puccinia in this country, C. Schœleriana might only be a variety

[1] Winter, 'Rabenhorst's Kryptogamen Flora,' 1881, vol. i, p. 182.
[2] Rostrup, loc. cit., p. 17 and p. v.

of it occurring upon C. arenaria. The following duplicated cultures were therefore made.

a. A quantity of P. Schœleriana was germinated in water in a watch-glass. This was divided into two parts, one of which was applied to a young plant of Senecio Jacobæa (260), and the other to a plant of Urtica dioica (261). The Senecio became affected with the Æcidium, but the Urtica did not.

b. Conversely, a quantity of P. caricis was germinated in water in a watch-glass, and divided into two parts, one of which was placed on a plant of Urtica dioica (258), and the other upon a Senecio (259). The Urtica became affected with the Æcidium, the Senecio did not. The spores of Æcidium Jacobææ applied to Carex arenaria gave rise to the Uredo (199, 388, 389). The teleutospores of Puccinia Schœleriana in seven separate cultures in every instance produced the Æcidium upon Senecio Jacobæa (260, 285, 291, 292, 293, 294, 447).

The Puccinia in question it is proposed to call Schœleriana after Schœler,[1] the Danish schoolmaster, who lived at the beginning of the present century in the village of Hammel, near Aarhuus, where he, by careful observation of what happened in nature, came to the conclusion that the yellow fungus on barberry has some connection with the rust on oats. He began these investigations in 1807, and continued them for some years.

In 1816 Schœler applied the "yellow dust" of the barberry fungus to some healthy rye plants, which were still moist with dew, and found the latter had, in the course of some few days, become badly affected with rust; "while at the same time not one rusty plant could be found anywhere else in the whole rye field." Schœler was also aware of the fact that rye became affected with rust without the intervention of the barberry.

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[1] Shœler, "Berberissens Skudelige Indflydelse pa Sæden," 'Landækom-miske Tidender,' 1818, part viii, p. 289.

Puccinia Schœleriana, n. sp.

I. Æcidiospores (Æcidium Jacobææ, Grev., 'Flor. Edin.,' p. 445), Æcidia in circular clusters, mostly upon the under surface of the radical leaves; cups with reflexed torn white edges; spermogonia upon the corresponding upper surface of the affected leaves; spores rounded, yellow, finely echinulate; 15μ to 20μ in diameter.

II. Uredospores upon yellow discoloured spots; sori elongate or rubrotund, surrounded by the ruptured epidermis; generally hypophyllous spores, subglobose or ovate, yellowish brown, rough; 25μ to 30μ long by 14μ to 20μ wide.

III. Teleutospores—Sori erumpent, oblong or elongate, large, prominent, almost black; hypophyllous naked, surrounded by the ruptured epidermis; spores on long, firm pedicels, slightly constricted; upper cell subglobose, ovate, or attenuated upwards; apex much thickened, rounded, or pointed; lower cell cuneiform, often paler than the upper; rich brown, smooth; 60μ to 80μ long by 15μ to 20μ wide.

No. of Expt.	Infecting Material.		Plant Infected.		Date of Infection.	1st Result
199.	Æcidium Jacobææ		Carex arenaria		21 June	15 July.
388.	,,	,,	,,	,,	31 May	12 June.
389.	,,	,,	,,	,,	31 May	20 June.
⎰ 258.	Puccinia caricis		Urtica dioica		16 Feb.	11 Mar.
⎱ 259.	,,	,,	Senecio Jacobææ		16 Feb.	—
⎰ 260.	,,	Schœleriana	,,	,,	17 Feb.	15 Mar.
⎱ 261.	,,	,,	Urtica dioica		17 Feb.	—
285.	,,	,,	Senecio Jacobææ		6 Apr.	30 Apr.
291.	,,	,,	,,	,,	6 Apr.	30 Apr.
292.	,,	,,	,,	,,	6 Apr.	30 Apr.
293.	,,	,,	,,	,,	6 Apr.	30 Apr.
294.	,,	,,	,,	,,	6 Apr.	30 Apr.
447.	,,	,,	,,	,,	15 Sept.	26 Sept.

Conclusions.—From the experimental cultures above it appears—

1. That Ranunculus repens is the host plant upon which both Uromyces poæ and Puccinia Magnusiana have their æcidiospores.

2. That these two Æcidia are not to be distinguished from each other anatomically.

3. That Ranunculus bulbosus is the host plant upon which both Uromyces dactylidis and Puccinia Magnusiana have their æcidiospores, which in like manner are anatomically indistinguishable.

4. That Uromyces poæ has its æcidiospores upon Ranunculus ficaria and R. repens.

5. That Uromyces dactylidis in this district has its Uredo without capitate paraphyses.

6. That the Æcidium upon Ranunculus acris belongs to the life cycle of Puccinia perplexans, a Puccinia the teleutospores of which occur upon Alopecurus pratensis, Avena elatior, and Poa sp. (?), bearing a close resemblance to those of P. rubigo-vera, but wanting the dark paraphyses of the latter species.

7. That the uredospores of P. perplexans are sometimes mixed with capitate paraphyses and sometimes without them.

8. That Puccinia phragmitis has its æcidiospores upon Rumex hydrolapathum, R. obtusifolius, L.; R. crispus, L.; R. conglomeratus, Mur., and Rheum officinale.

9. That P. Magnusiana has its æcidiospores upon Ranunculus repens and R. bulbosus.

10. That the Æcidium upon Rumex acetosa is neither connected with P. Magnusiana nor with P. phragmitis.

11. That the Æcidium on Senecio Jacobæa belongs to the cycle of a Carex inhabiting Puccinia—P. Schœleriana.

	Æcidiospores.	Uredo and Teleutospores.
Uromyces poæ, Rhb.	Ranunculus repens 　　　　　" ficaria	Poa trivialis. 　" pratensis.
Uromyces dactylidis, Oth.	Ranunculus bulbosa	Dactylis glomerata.
Puccinia Magnusiana, Korn.	Ranunculus bulbosa 　　　　　" repens	Phragmitis communis.
Puccinia perplexans, Plow.	Ranunculus acris	Avena elatior. Alopecurus pratenses. Poa sp.?
Puccinia phragmitis, D. C.	Rumex obtusifolius 　" hydrolapathum 　" crispus 　" conglomeralus Rheum officinale	Phragmitis communis.
Puccinia Schœleriana Plow.	Senecio Jacobæa	Carex arenaria.

On the Occurrence of Chitin as a Constituent of the Cartilages of Limulus and Sepia.

By

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THE question of the chemical composition of the cartilage that occurs in various invertebrate animals does not seem to have been the object of previous research.[1] In an article by Professor Lankester on "The Skeleto-trophic tissues of Limulus," which appeared recently in this Journal,[2] he states that Professor Schäfer having, at his request, chemically examined the ento-sternite of that animal, had found that chitin was probably present in that organ. Professor Lankester subsequently placed a larger supply of this tissue in my hands for the purpose of determining the presence of chitin; in addition I have, at Professor Lankester's suggestion, submitted to a similar examination the cartilage found in the head of the cuttle-fish (Sepia).

These cartilages are in appearance similar to that found in vertebrate animals; but chemically they are different, as they contain chitin in addition to a chondrin-like body.

We may consider the subject under the following heads:

    A. Composition of the Cartilage of Sepia.

    B. Composition of the Entosternite of Limulus.

    C. Existence of Chitin in the Liver of Limulus.

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[1] In his paper recently published on the subject of cartilage, Krukenberg makes no reference to the cartilage of the two animals I have examined. Krukenberg, "Die chemischer Bestandtheile des Knorpels," 'Zeitschrift für Biologie,' xx Band, 3 Heft, München und Leipsig, 1884.

[2] 'Quarterly Journal of Microsc. Science,' Jan., 1884.

A.—Composition of the Head Cartilage of Sepia.

The specimens from which the cartilage was taken had been preserved for some time in spirit. After having been soaked in water for twenty-four hours to remove the spirit, it was divided into small pieces, and various portions were treated in the following ways:

1.—Some was boiled with distilled water in a sealed tube for many hours. Apparently no change took place in the cartilage. After this time the water was poured off, and tested as follows :

    a. It did not gelatinise on cooling.

    b. It contained no evidence of proteids.

    c. Acetic acid gave no precipitate. This showed that no mucin had dissolved in the water.

    d. Solution of tannin, acetic acid, and ferrocyanide of potassium, lead acetate, and mercuric chloride, all gave small amounts of precipitate.

2.—Hydrochloric acid was added to another portion. In a few hours in the cold, the cartilage was completely dissolved; on adding water to this solution, a small amount was reprecipitated.

3.—Acetic acid was added to a third portion. The cartilage swelled up somewhat, but underwent no further change.

4.—To a fourth portion Baryta water was added. The cartilage became dissolved to some extent; and on adding acetic acid to the solution, a precipitate was obtained.

5.—Sulphuric acid was added to another portion. The cartilage turned of a brownish hue, but did not dissolve until the application of a small amount of heat, when a dark brown solution was formed. On diluting this, and heating it for half an hour in boiling water, it was found to possess the property of reducing copper salts.

6.—To a sixth and last portion, strong solution of caustic potash was added. The cartilage became in the course of a few hours disintegrated, and to some extent dissolved; but there was a considerable amount of residue which was not lessened by boiling. The solution was found to contain no sulphur.

The conclusions to be drawn from these reactions are as follows :

1. Elastin is absent, because the cartilage is wholly soluble in cold concentrated hydrochloric acid.

2. Keratin is absent, because the part soluble in potash contains no sulphur ; and because acetic acid does not dissolve anything from the cartilage.

3. Mucin is present, as is shown by acetic acid causing a precipitate when added to the solution in Baryta water.

4. Gelatin, if present at all, is present in very minute quantity, as is shown by tannin producing a precipitate in the watery solution; but not present in quantity sufficient to cause gelatinisation.

5. The basis of the cartilage is a substance soluble in alkalies. Chondrin is now regarded[1] by many as merely a mixture of mucin and gelatin; it would seem that this is what we have here; the tests for both these bodies can be obtained; that the gelatinous element is, however, present to a slight extent only is shown by the fact that gelatinisation does not take place; or this latter fact may be due to the coexistence of chitin mixed with it.

6. That in addition to this chondrin-like body, the cartilage contains a body insoluble even in boiling alkalies. This residue after boiling with potash will presently be shown to consist of chitin.

7. Little can be concluded from the fact that a sugar-like body reducing copper salts can be obtained by boiling with dilute sulphuric acid, since chondrin, mucin, and chitin all behave in this way.

We have next to consider the composition of the residue left after boiling with solution of potash.

A large quantity of the cartilage was taken, potash added; the residue, a colourless amorphous body, collected, and washed thoroughly by decantation with distilled water. It was divided into two parts, which were treated in the following way:

1. One part was dissolved by adding hydrochloric acid to it; a clear solution was formed. This solution gave the following tests:

a. On adding water it was reprecipitated.

b. Another portion was treated in a water-bath for about an hour. The colour of the solution became brown. On evaporating to dryness crystals of a brownish hue were formed;

[1] Mowchowitz, "Zur Histochemie des Bindegewebes," 'Verhandl. d. Naturhist. Med. Vereins zu Heidelberg,' Vol. i, Part 5.

portions of these were allowed to crystallise on a glass slide, and then examined with the microscope; they were found to present the following appearances:—The crystals were single, and also in clusters and star-shaped masses. They varied in size considerably; the average length was ·07 to ·08 mm., and the breadth varied from ·01 to ·001 mm.

At first sight the star-shaped clusters reminded one of leucin or tyrosin, but closer investigation showed that they were not composed of these materials. They had a slightly brownish tinge, and the crystals were oblique rhombic columns. Some of these were so thin that they lay flat on the slide as rhombic plates; the angles of these rhombs were measured by means of a goniometer stage attached to the microscope. The acute angle was found to be very acute, being on the average $39° 25'$, the obtuse angle being therefore $140° 35'$.

They did not polarise light.

They were readily soluble in water, soluble with difficulty in alcohol. From the alcoholic solution they could be recrystallised. The crystals so obtained were slenderer, and had lost their brownish tint.

2. The other part was dissolved by adding hydric sulphate. This solution was diluted and boiled for half an hour; it was then found to have the power of reducing copper salts.

We have now data amply sufficient for the identification of this body. It is, in fact, chitin.

It will be here convenient to enumerate the properties of chitin as at present known, and afterwards to point out the resemblances between it and the body obtained from the cartilage of Sepia.

The Properties of Chitin are as follows:—It is a white amorphous body, insoluble in water, in weak acids, and in boiling concentrated alkalies; soluble in strong acids. When dissolved in sulphuric acid it yields a body which reduces copper salts. This was supposed by Berthelot to be a fermentable sugar,[1] but the researches of Ledderhose[2] have shown

[1] Berthelot, 'Comptes Rendues,' xlvii, 227.
[2] Ledderhose, "Ueber Chitin, und seine Spaltungsprodukte," 'Zeitschrift

that this is really a nitrogenous body—glycosamine—having the formula, $C_6H_{13}NO_5$.

When dissolved in hydrochloric acid the solution of chitin is colourless, and chitin can be precipitated from this solution unchanged by the addition of water. When[1] the solution is boiled it becomes black in consequence of a decomposition, which is completed in about an hour. On evaporation impure hydrochlorate of glycosamine is obtained, and is purified by recrystallising repeatedly. This body is easily soluble in water, soluble with difficulty in alcohol, and reduces alkaline solutions of cupric and silver salts.

Professor Gamgee kindly sent to Professor Lankester some crystals of this salt, which he had prepared from lobsters. I also prepared some from the exoskeletons of cockroaches.

The naked-eye examination of these crystals showed them to belong to the monoclinic system.[2]

The angles were, however, not perfect; the crystals were consequently redissolved in water, and allowed to recrystallise therefrom on a glass slide. They were then submitted to microscopical examination.

In the case of the salt prepared from cockroaches the following are the results obtained: in the impure state they have a light-brownish tinge, which they lose after recrystallisation. Their form is that of flat parallelograms, as in the case of the crystals prepared from Sepia, and these are sometimes in clusters. Measurement of the acute angle of the parallelogram gave on the average 39° 25′.

The crystals had no action on polarized light.

In the case of the salt prepared by Professor Gamgee from lobsters, redissolved in water, and allowed to recrystallise on a

für Physiol. Chem.,' vol. ii (1878), p. 213, and " Ueber Glykosamin," ibid., vol. iv (1880), p. 139.

[1] Gamgee, 'Physiological Chemistry,' p. 301.

[2] The crystals which I had the opportunity of examining were about half an inch long; Professor Gamgee states that with plenty of material he can obtain crystals several inches in length, and of proportionate width.

slide, the following were the results obtained : they were colourless,[1] and their form was that of flat rhombic columns radiating from various centres ; measurement of the acute angles of some of the more perfect of these gave as an average 39° 25′.

The crystals had no action on polarized light.

Having thus seen the properties of the substance obtained from the cartilage of Sepia, and that generally known as chitin occurring in the exoskeleton of insects, Crustacea, and other invertebrates, we can proceed to compare them; and can do so most readily by means of the following table :

	Substance prepared from Cartilage of Sepia.	Substance prepared from Chitin.
Condition.	Amorphous, white.	Amorphous, white.
Action of water . . .	Insoluble.	Insoluble.
Action of weak acids .	Insoluble.	Insoluble.
Action of boiling alkalies	Insoluble.	Insoluble.
Action of hydrochloric acid (in the cold)	Soluble : reprecipitated by adding water.	Soluble : reprecipitated by adding water.
Action of sulphuric acid	Soluble : the solution reducing cupric salts	Soluble : the solution reducing cupric salts
Prolonged action of hot hydrochloric acid	The solution becomes brown, and a crystalline substance can be obtained from it.	The solution becomes brown, and a crystalline substance (hydrochlorate of glycosamine) can be obtained from it.

That the crystalline substance obtained from the cartilage of Sepia is really hydrochlorate of glycosamine is seen by studying its properties, under the following heads :

Its crystalline form ; including the measurement of its angles ; the very acute angle is quite characteristic.

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[1] In more impure crystals sent by Professor Gamgee, the same light-brown tinge was noticed, as in those prepared by me from cockroaches.

Its action upon polarized light.

Its ready solubility in water.

Its slight solubility in alcohol.

Comparing the properties of the crystals under these four heads, we find them to be similar in every respect; the irresistible conclusion is, therefore, that the crystalline substance obtained from the cartilage of Sepia is hydrochlorate of glycosamine, and that the cartilage of Sepia contains chitin.

The question now remains, how much chitin does this cartilage contain ? the method I have adopted in the quantitative analysis has been the following :

A known weight of cartilage is taken, and potash added to it ; the residue is washed, collected on a dried and weighed filter ; it is then dried at 100° C., and weighed ; the increase in weight gives the weight of the precipitate in the dry form, from this the amount of ash is deducted, and from the remainder the percentage can be calculated.

The average of two such quantitative experiments gives the percentage of chitin in the cartilage of Sepia as 1·22.

B.—Composition of the Entosternite of Limulus.

What has been said for the cartilage of Sepia may be repeated in very good measure for the cartilaginous Entosternite of the king-crab. The method of analysis was the same, and the results are as follows :

The greater part of the ground substance is composed of a chondrin-like body, giving the tests for mucin, and to some extent also those for gelatin (viz. precipitation by tannin, lead acetate, mercuric chloride, ferrocyanide of potassium, and acetic acid) ; but not sufficient gelatin is present to cause gelatinization to occur in cooling the hot watery solution.

Keratin and elastin are absent.

Chitin is present : this is shown by—

1. There is a residue insoluble in boiling alkalies, soluble in cold concentrated hydrochloric acid; the addition of water reprecipitating it from its solution.

2. By boiling it with sulphuric acid a body which reduces cupric salts is formed.

3. By boiling it with hydrochloric acid, crystals of hydrochlorate of glycosamine are obtained.

The percentage of chitin present (the average of three analyses) is 1·01.

It should be here mentioned that I performed some control experiments with the cartilage of two vertebrate animals, viz. the cat and rabbit, taking the rib cartilages in each instance; but in neither was there any residue after boiling with concentrated caustic potash.

The cartilage, then, of the two invertebrate animals I have examined differs in a very important way from that of vertebrates; namely, in containing chitin in its composition.

C.—The Existence of Chitin in the Liver of . Limulus.

The following analysis is merely a qualitative one, and shows conclusively, that chitin is present in the liver of Limulus, though whether actually in the liver-cells, or in the connective tissue of that organ, which is very abundant, I am unable to say. It seems more probable that the latter is the correct view.

The livers of four king-crabs, which had just been killed, were digested with a large amount of caustic potash for three or four days. After this time most of the constituents of the liver were dissolved, but there was a considerable amount of insoluble residue. This was filtered off. The filtrate was brown, and perfectly clear; the residue was also of a brown colour, thick, muddy, and partially flocculent. The residue was collected, washed with water, and again digested with potash of the same strength; it was thus obtained of a lighter colour; by repeating the process, almost colourless flocculi were obtained. It (the residue) was then washed with distilled water and found to be insoluble in boiling water, and also in concentrated boiling potash. It was soluble in concentrated

hydrochloric acid in the cold, from which solution it was reprecipitated by the addition of water in the form of white, colourless flocculi.

It was also soluble in concentrated sulphuric acid ; this was diluted and boiled, and then was found to possess the property of reducing cupric salts.

These preliminary tests clearly pointed to the body being chitin ; its solubilities and insolubilities are of themselves almost characteristic of this substance.

The indication was rendered a certainty by boiling the colourless solution in hydrochloric acid ; in about half an hour it became dark brown, owing to the formation in it of the hydrochlorate of glycosamine as in the previous cases.

Till now the generally received opinion has been that chitin occurs solely in epiblastic structures ; Ewald and Kühne[1] found a body resembling it in the nervous system of Crustacea, but this also is epiblastic.   In the nervous system of these animals, it seems to replace what Kühne calls neuro-keratin, a horny substance occurring in the nerve-fibres of vertebrate animals. But it is clearly not confined to the epiblast ; for in three instances chitin has been now shown to occur in mesoblastic structures, viz. in the cartilage of the cuttlefish and king-crab, and in the liver[2] of the latter animal.

[1] Ewald u. Kühne, " Ueber einem neuen Bestandtheile d. Nervensystem " ' Heidelberg Verhandlungen,' 1877.

[2] If the chitin, present in the liver, is in the liver-cells, not in the connective tissue as above supposed, we have an instance of chitin occurring in hypoblast.

The Urinary Organs of the Amphipoda.

By

W. Baldwin Spencer, B.A.,
Scholar of Exeter College, Oxford.

With Plate XIII.

WITHIN the group Arthropoda very various structures are met with subserving the function of excretory organs.

The most primitive are undoubtedly nephridia as yet known only to exist in one member — Peripatus, whilst the most generally occurring are the Malpighian tubes whose presence is highly characteristic of and possibly confined to the Tracheata; indeed, this is usually considered a not unimportant point of difference between the latter and the Crustacea.

Notwithstanding this it is known that in certain Crustaceans there do exist small but well-defined appendages opening into the posterior part of the alimentary canal, though whether into the mid or hind gut is a disputed point.

In English text-books, with, so far as I am aware, but one exception, their existence is either passed over in silence or merely mentioned, whilst nothing definite is stated with regard to their nature and function.

The one exception is the work of Messrs. Bate and Westwood on 'Sessile-eyed Crustacea.' Here their presence is fully recognised and a somewhat detailed description is given, though the power of cutting continuous sections enables us now to study their structure and relations more accurately than it was then found possible to do.

At the suggestion of Professor Moseley, to whom I am indebted for advice and the material necessary for the work, I have investigated these organs in two or three typical forms, and have, moreover, attempted in the following article to give a short summary of what appears to be known concerning their existence and nature in the various specimens in which they are found.

Within the Crustacea they are apparently confined to the Edriophthalmata, whilst even amongst these their range is limited as they are not known to exist in the Isopoda. I have cut continuous transverse sections through the marine from Idothea, the fresh-water Asellus, and the terrestrial Oniscus, but in none of these are there present any appendages opening into the hinder part of the alimentary canal. In the Amphipoda and in Caprella, on the other hand, there is no difficulty in demonstrating their existence.

Of English authors, Huxley,[1] whilst treating of the Edriophthalmata, merely says, " Occasionally there are one or two cæca which open into the posterior part of the intestine and appear to be urinary organs analogous to the Malpighian cæca of insects."

Gegenbaur[2], speaking of the appendages of the hind gut in Arthropoda, says, " In the Crustacea we sometimes meet with cæcal organs on the hind gut, as, for example, in the larvæ of the Copepoda, but we cannot safely form any opinion as to their significance," whilst he makes no mention of the organs in Amphipoda.

Balfour,[3] also in his summary of arthropodan development, says, " The derivation of the Malpighian bodies from the proctodæum is common to most Tracheata. Such diverticula of the proctodæum are not found in Crustacea." These particular appendages, as well as the manner of their development, are unnoticed by him.

Various foreign investigators have dealt with the subject in

[1] 'Anatomy of Invertebrated Animals,' p. 364.
[2] 'Elements of Comparative Anatomy,' English translation, p. 276.
[3] 'Comp. Embryology,' vol. i, p. 452.

considerable detail, and their works will be referred to in the descriptions which follow.

The presence of these tubes in Gammarus was described by Sars, who, judging apparently from their position only, came to the conclusion that they were outgrowths of the hind gut, and analogous to the Malpighian tubes of insects. In both young and old specimens of Gammarus their existence may be demonstrated either by simple dissection or best by cutting continuous sections through the whole animal. Even in very young Gammari, which have just left the brood pouch of their mother, they form prominent objects. Thus, in one of 2 mm. length they arise in the third segment from the posterior end and pass forwards, lying dorsad of the alimentary canal through four segments, growing to a slightly greater length in the adult.

To observe their structure and position with regard to the other organs, the best method, as before said, is to cut sections through the whole body. Fig. 3 represents one near the posterior end cut somewhat obliquely, so that only the actual opening of one of the tubes into the alimentary canal is seen. It will be observed that the epithelium lining both tubes and canal is similar and continuous, and that the former arise quite separately from each other on the dorsal surface.

Below the canal the four liver tubes are seen cut in section, and below these again the nerve cord with ganglion cells on its ventral, and fibres on its dorsal side.

A more anterior section shows that the two tubes lie closely side by side, each surrounded, as are the liver cæca, by a definite membrane, whilst the alimentary canal is supported by a well-marked mesentery which in this part divides the body cavity into two halves, a dorsal and a ventral.

If a fresh specimen be taken the two tubes are seen to have their walls composed of long cells rounded internally (i. e. the end of each cell projects slightly into the lumen of the tube), whilst externally they are roughly hexagonal in shape. When stained they show large nuclei lying always on their outer side.

The tubes pass forward, retaining the same position, until having traversed five segments they end blindly.

The only change in their position is produced by the development of reproductive products which lie between the tubes and the alimentary canal, and from which, though in very close contact, the former may be seen to be separated by a distinct membrane. Fig. 5 shows diagrammatically the relative positions of the organs of Gammarus pulex.

These organs in the Gammaridæ are treated of in considerable detail by Nebeski[1] in his account of the Amphipoda, and he describes them as being present in very varying stages of development in different members. In Melita a single and very rudimentary one is present. Corophiiden and Mœra each possess a pair of small ones, whilst in Gammarus and Cyrtophium the pair are much better developed. In Orchestia not only were the tubes still more prominent, but he found within them what he states are excretory products in the form of concretionary bodies, each of which takes its rise within an epithelium cell of the tube, and, gradually growing, pushes the cell substance aside and comes to lie within the lumen. These bodies, which appear from his description to exactly resemble in form others which I have found in the closely allied Talitrus locusta, Nebeski states consists of calcium carbonate.

Gamroth[2] has described the presence of the tubes in the Caprellidæ, and states that though their intimate structure and physiological import is unknown to him, yet he has found granular concretions in them, and regards them as excretory organs. When transverse sections of the hinder part of Caprella are cut their existence is very easily recognised, as they form very prominent pouches lying one on either side of the walls of the alimentary canal communicating with the latter by somewhat constricted openings. Nothing apparently enters these sacs (for such they are in form in Caprellidæ rather than tubes) from the gut, as in sections in which the latter is full of food the sacs themselves are quite empty. Their walls consist of elongate nucleated cells, just as in Gammarus and Talitrus.

[1] 'Beitrag zur Kentniss der Amphipoden der Adria,' Zool. Institute, Wien Band iii, 1881.

[2] 'Zeitschr. f. Wiss. Zool.,' Bd. xxxi, p. 115.

Mayer[1] has also described them in the Caprellidæ, where he states that they are well developed in Caprella, and absent, or only very feebly developed, in Protella, Proto, and Podalirius, but when present he has never found in them characteristic concretions, and is very decided in asserting that throughout the Amphipoda these diverticula, whatever may be their function and whether they contain excretionary products or not, belong morphologically to the mid and not to the hind gut, and that hence they cannot be considered as analogous to the Malpighian tubes of insecta. He states that there is always present a sharp break in the epithelium where the mid and hind gut meet, and that the chitin lining of the latter is not continued into the tubes whose epithelium resembles that of the mid, and not that of the hind gut.

I have lately carefully investigated the nature of these tubes in numerous specimens of Talitrus locusta, where they may without any difficulty be discerned by carefully removing from the animal the whole of the alimentary canal, and after laying this out upon a slide gently separating the two tubes from the side of the hind gut close to which they lie though clearly distinguishable by their whitish colour.

Fig. 1 represents part of the alimentary canal of Talitrus which has been removed from the body with the tail segment still attached, though the liver tubes when in the body would lie in the contrary direction. The figure is drawn to scale, and shows the relative length of the parts.

When compared with fig. 5 of Gammarus a point of considerable difference between the two is seen at once, the tubes in Talitrus opening at a considerable distance from the anus and running backwards instead of forwards, as in the former, to end blindly in the last segment. They are, indeed, very similar to the Malpighian tubes of insects, more especially resembling those of such a form as Julus, where only one pair is present.

Fig. 2 represents a transverse section through the hind gut of Talitrus in which the two tubes are seen lying some distance

[1] 'Die Caprelliden des Golfes von Neapel,' p. 147.

apart from each other, not closely applied upon the dorsal surface of the alimentary canal as in Gammarus, whilst also their openings into the gut are lateral and not dorsal. The definite shape of the hind gut may be noticed in passing, as also the fact that it possesses a distinct cuticular lining beset throughout the greater part of its course with little processes, though towards the anterior end these disappear, and the cuticular lining itself becomes very thin indeed.

With regard to the tubes their walls are cellular in nature just as in Gammarus, and by focussing under a high power each cell may be seen to have its inner end which faces into the lumen of the tube rounded, whilst its outer end is roughly hexagonal in outline. The most interesting fact, however, is that, in certain specimens, as in the one figured, these tubes were found to contain very definite concretions. If a great number of animals be examined there will be found perhaps one or two of a light greenish colour, and through the cuticle of which may be seen, on either side posteriorly, a white streak indicating the position of these tubules. In a specimen of this description concretions will most likely be found filling the whole cavity of the tube.

The concretions are of various sizes and arranged as in the figure, smaller ones being placed between each larger one, the latter, apparently consisting of several of the former united together in some manner. In no case could any sign of a concretion be observed either within or between the cells of the tubes themselves.

In one or two instances they have been met with in an animal of a dark reddish-brown colour, differing in tint from the usual greenish brown, though in this case the concretions were only small ones and did not exist in the proximal, but only in the distal half of the tube.

Though as yet I have not been able to obtain definite proof, still, judging from the condition of the cuticle in the two kinds of specimens in which only these concretions have been found (the first mentioned especially being clearly distinguishable from the normal Talitrus), it may not appear unjustifiable to

suggest that these concretions have something to do with the process of "casting the skin," and that the first mentioned were animals in which this had just taken place, whilst the second were those in which preparation for it was being made.

The concretions are, of course, extremely minute, and have only been obtained from a few specimens, so that it is not easy to determine exactly their nature. Distilled water does not dissolve them, nor is there any uric acid present, but I have been able to clearly detect phosphoric acid, and hence they seem to differ from those found by Nebeski in Orchestia cavimana, where he states that they consist of carbonate of lime.

It has not been possible to observe what becomes of the concretions, but in one of the specimens mounted a few of them, whether accidentally or not I cannot say, have passed out into the alimentary canal by means of the mouth of the tube, showing, at all events, that it is perfectly possible for them to do so.

At first sight it might appear as if these tubes were homologous with the Malpighian tubes of Tracheata, and until their development has been worked out and compared with that of the latter, it is impossible to settle the question definitely. In all specimens they arise from the point of junction of the mid and hind guts, but on close inspection and by means of sections, it can be demonstrated clearly, in at all events certain cases, that they belong really to the mid gut.

Thus fig. 4 represents a longitudinal vertical section through the hinder part of the alimentary canal of Gammarus pulex. One of the tubes is seen arising on the dorsal surface, but its lining epithelium is clearly continuous directly with that of the mid gut, whilst there is seen to be a distinct break (fig. 4, x) where the latter ceases and the hind gut begins.

These organs, which have such a strangely limited distribution amongst Crustacea are certainly, as is proved by their products, excretionary, and are very probably also urinary in function, but the knowledge which we at present possess of their point of origin from the alimentary canal prevents us

from regarding them as strictly homologous with the Malpighian tubes of Tracheata.

EXPLANATION OF PLATE XIII.

Illustrating Mr. W. B. Spencer's paper on "The Urinary Organs of Amphipoda."

List of Letters employed.

An. Anus.   *Cr.* Concretions in urinary tubes.   *Cr'.* Concretions which have passed from the tubes out into the hind gut.   *G.* Genital organs.   *H.* Heart.   *H. g.* Hind gut.   *L.* Liver tubes.   *Lo.* Opening of liver tubes into mid gut.   *M. g.* Mid gut.   *Mes.* Mesenteries supporting and surrounding the various organs.   *M. lg.* Longitudinal muscular fibres of hind gut.   *M. tr.* Transverse muscular fibres of hind gut.   *Musc.* Ordinary muscles of the body. *N.* Nerve cord.   *R.* Rectum.   *Sg.* Last segment of the body removed with the gut.   *Ur.* Urinary tubes.   *Ur'.* Distal part of urinary tube, which is always bent forward.   *Ur. o.* Opening of urinary tubes into gut. *X.* Point at which hind gut ends and mid gut begins.   *Y.* Anterior termination of mid gut.

Fig. 1.—Part of alimentary canal of Talitrus locusta, removed from the body with the tail segment still attached. Only the mid and hind guts are present and the liver tubes, only one of which is shaded, the rest being only drawn in outline are turned forwards. The two urinary tubes are seen arising from the posterior end of the mid gut ; they have been pulled away from the side of the hind gut, close to which they lie naturally ; the constantly bent distal part lies normally in the last segment. Large and small concretions are seen filling up the tubes, some having passed out into the gut.

Fig. 2.—Transverse section through hinder part of body of Talitrus locusta. The hind gut has a distinct cuticular lining beset with small points. Both urinary tubes are seen in section, each enclosed by a supporting mesentery, as is also the gut. Below the latter the nerve cord is seen in section.

Fig. 3.—Transverse section through hinder part of Gammarus pulex. The plane of the section is oblique, so that the opening of only one urinary organ into the mid gut is seen ; the cells of the other one are also cut through,

though no lumen is seen.   In consequence of the obliquity of the section the liver tubes also are only cut through on one side.

FIG. 4.—Longitudinal vertical section through part of the alimentary canal of Gammarus pulex.   The opening of one of the urinary tubes is seen, and the continuity between the epithelium of the latter and that of the mid gut.   At the point X is seen the clear termination of the mid gut, and commencement of the hind gut.   The muscular fibres of the hind gut are seen in section.

FIG. 5.—A diagrammatic representation of the internal organisation of Gammarus, showing the relative position of the urinary tubes.
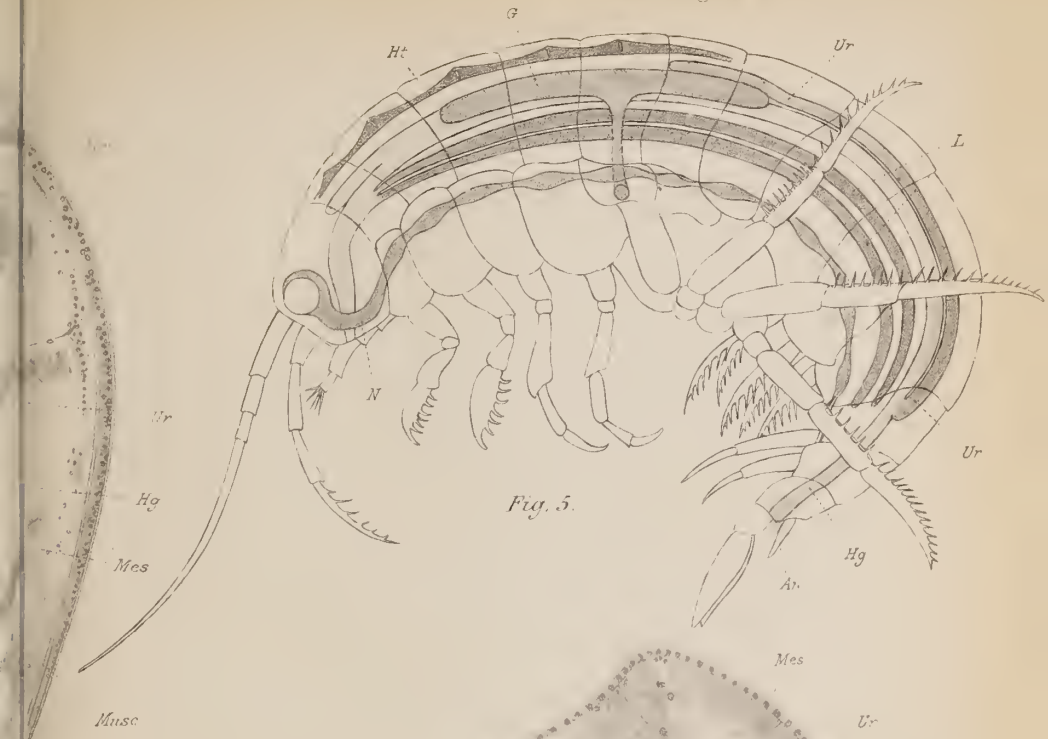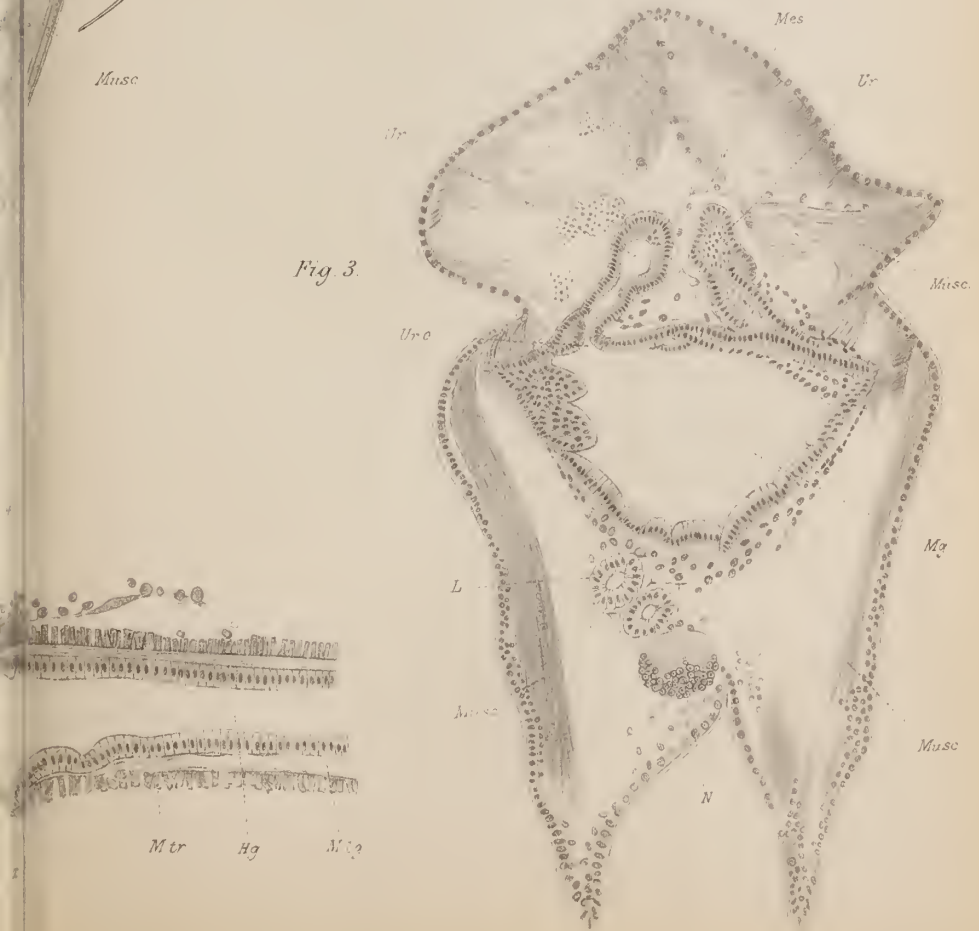
Fig. 1.

Fig. 2

Fig. 4.

Fig. 5.

Fig. 3.

On the Skin and Nervous System of Priapulus and Halicryptus.

By

Robert Scharff, Ph.D.

With Plate XIV.

DURING the past year I have had the opportunity of re-investigating the anatomy and histology of Priapulus cau-datus and Halicryptus spinulosus from specimens supplied by the liberality of my esteemed teacher Professor Bütschli, by whose help and advice I have greatly profited, and to whom my most cordial thanks are due.

I first intended to give a full account of the anatomy of these two interesting forms, but the results I obtained agree in general with those published by Ehlers in his valuable paper " Ueber die Gattung Priapulus." Histologically, how-ever, he left a good deal to do for later observers, and although Horst as well as Saenger have extended our knowledge con-siderably in that respect, a description of the nervous system and skin, dealing especially with its peculiar dermal organs, probably of a sensory nature, may be of interest.

THE SKIN.

According to Ehlers[1] we have to distinguish two layers in

[1] E. Ehlers, " Ueber die Gattung Priapulus," 'Zeitschrift für wissenschaft-liche Zoologie,' vol. xi, p. 223 ; ditto, " Ueber Halicryptus," loc. cit., vol. xi, p. 404.

the skin of Priapulus caudatus and Halicryptus spinu-losus, viz. a cuticula and a subcuticular layer or hypodermis.

To these two layers I have to add another extremely thin one consisting of connective tissue (fig. 8, d.). It does not reach half the thickness of the cuticula in the proboscis, while in the body or trunk it is still thinner, and could only be clearly demonstrated in a few places.

In Sipunculus nudus this cutis is well developed, and is the seat of secreting glands and accumulations of pigment.

The Cuticula.

The cuticula covers the whole body, varying in thickness in the different parts. At the oral aperture it turns in, coating the interior of the œsophagus. On the body, especially at the posterior portion of that of Halicryptus, the cuticula reaches a considerable thickness.

It is composed of two parts (figs. 1, 2, 3), an external thin homogeneous layer (ce.) and an internal thicker one (ci.) also lighter in colour, which is distinctly stratified. In cutting sections for the microscope it often happens that the whole of the cuticula or some of the outer layers split off, and thus show undoubtedly that it is made up of distinct strata. Reticular markings are seen on the surface of the cuticula on the proboscis. They are generally in shape of polygonal figures and correspond to the cells of the hypodermis below, which have the same form in a surface view, and from which the cuticula has been secreted.

According to Horst[1] the cuticula of the so closely-allied Priapulus bicaudatus appears to possess a honey-combed structure, which is produced by two systems of vertical lamellæ crossing one another, dividing it into a series of prisms. The same writer agrees with Ehlers as to the cuticula of Priapulus being insoluble, while that of Sipunculus nudus has by Andreae[2] been shown to be soluble in boiling caustic potash.

[1] Horst, "Anatomie von Priapulus bicaudatus," 'Niederl. Archiv f. Zoologie,' Supplement to vol. i, p. 16.

[2] Andreae, "Beiträge zur Anatomie und Histologie des Sipunculus nudus," 'Zeitschr. f. wiss. Zoologie,' vol. xxxvi, p. 207.

Ehlers designates the cuticula of Priapulus as " chitin ;" some investigators, on the other hand, like Graber,[1] seem to suppose that its identity with the chitin of the Arthropoda is as yet very doubtful.

The Hypodermis.

The hypodermis in its various modifications forms the substratum to the cuticula. It consists generally of a thin layer of about the same thickness as the cuticula. Looking at the hypodermis from above it appears as a layer of polygonal cells corresponding to the markings found on the cuticula. A cross section reveals the shape of the cells, which contain only a small quantity of protoplasm, and a comparatively large nucleus (figs. 1, 2, 3, h.). They send out numerous processes on the inner surface, by means of which the cells seem to communicate with one another.

On the proboscis we find longitudinal rows of little spikes or cones projecting from the skin, which will be described later on under the head of sensory organs. Their interior is stocked with hypodermic cells, which have been drawn out in shape of very long threads. In the small papillæ on the body of Priapulus a similar elongation of the cells takes place, while in those of Halicryptus they are still further modified into three different groups, showing an important diversity of form (figs. 3 A, 3 B).

Round about the anus the hypodermis of Priapulus caudatus undergoes a curious modification (fig. 4) in distinction to Priapulus bicaudatus and Halicryptus, where this does not occur. The cells become very much elongated, but at the same time they expand also in width so as to form a compact mass (fig. 10), and their protoplasmic contents increase, filling up the whole of the cells.

Ehlers describes these cells " as a heap of glandular bodies lying under the subcuticula," and Graber[2] says " Bei geeigneter

[1] Graber, " Ueber die Haut einiger Sternwürmer," ' Berichte d. Kais. Ak. d. Wiss.,' vol. lxvii, p. 61.

[2] Graber, loc. cit., p. 64.

Behandlung kann man über die Natur dieser Körper nicht lange im Zweifel sein. Es sind, um es kurz zu sagen, räumlich differencirte Theile einer am Stammesende mächtig entwickelten Cutis."

As I shall attempt to show further on, it is extremely probable that we have here to deal with secretory organs.

The hypodermis as well as the cuticula are also found on the peculiar respiratory appendage of Priapulus (fig. 11, resp.) the so-called " Schwanzanhang ;" the two layers, however, become very thin.

It now remains to consider the modifications of the hypodermis on the ventral surface of the body where the nerve-cord runs. An interesting transformation of hypodermic into ganglionic cells may be seen here, their nuclei swelling up and the rest of the cells becoming considerably attenuated. A look at fig. 6 and 7, however, will help to elucidate this better than a long explanation.

Horst[1] describes the hypodermis of Priapulus bicaudatus "as a thin layer, the thickness of which in the trunk only amounts to 0·003 mm." "It is composed of small branching cells with pretty large nuclei and only little protoplasm. The cellular bodies are connected with one another by numerous slender processes, and thus they assume a reticulated appearance."

The Cutis.

As already mentioned the existence of a cutis is extremely difficult to demonstrate, at any rate in the body. In the proboscis it becomes more evident, and consists of a very thin layer of fibrous connective tissue (fig. 8, d.). It is especially well seen where the longitudinal muscles are attached to the body wall, the hypodermis bending somewhat towards the interior, and the cutis attaching itself to the muscle.

This cutis corresponds as regards position to Keferstein's[2] " gestrichelten Haut," which he found underlying the hypo-

[1] Loc. cit., p. 17.
[2] Keferstein, ' Untersuchungen über niedere. Seethiere,' p. 41.

dermis in Phascolosoma puntarenæ and antillarum. A far greater development of the cutis is to be found in Sipunculus, which, according to Andreae,[1] consists mainly of areolar tissue, and in which secreting glands and pigment cells appear to be embedded.

Sensory and Secretory organs of the Skin.

In the contemplation of the sensory and secretory organs we shall have to consider Priapulus caudatus and Halicryptus spinulosus separately, there being some difference between the two with regard to these organs. I shall first mention the general disposition of these organs on the body, and then give a description of their form, histological structure, and probable function, finishing up with a few remarks as to their relation to similar organs in other animals.

On the proboscis of both of them the small dermal projections or "spikes" are arranged in numerous longitudinal rows which are more regular in Priapulus than Halicryptus. On the body or trunk on the other hand there are no longitudinal but only circular rows of spikes. The spikes are here so disposed as to occupy a median position on each annular muscle, and thus we get a series of parallel rings of them. In Halicryptus the spikes are similarly disposed to the end of the body, two larger spikes standing on each side of the anus (see Sänger,[2] pl. x, fig. 6). In Priapulus irregular masses of papillæ are scattered about at the posterior end of the body (the ordinary spikes being here absent) with the exception of the immediate surroundings of the nerve-cord on the ventral surface (fig. 4, *p.*). Little spikes also occur on the respiratory appendage.

[1] Loc. cit., p. 208.

[2] Saenger, "On Halicryptus spinulosus and Priapulus caudatus," 'Transactions of the 2nd Congress of Russian Naturalists in Moscow, 1869.' (Written in Russian.)

Priapulus caudatus.

On the proboscis the spikes are in form of small truncated cones, just visible by the naked eye. It has been mentioned before that the hypodermic cells which constitute the interior of these cones undergo a transformation, becoming very much elongated (fig. 1, *h.*), and converge somewhat towards the centre of the base. The cuticula forms the outer layer of the cone, and leaves a circular opening at the top. Through this opening a number of small delicate hairs are seen to project, piercing a thin membrane (*m.*) which covers the distal ends of the cells. The outer darker layer (fig. 1, *ce.*) of the cuticula does not quite reach the apex of the cone, but only extends about half way up and surrounds it as a sort of a sheath.

Various circumstances have led me to believe that the upper part can be retracted into this sheath, although I never examined the animal in the living state. Sometimes I met with sections in which the upper part was actually partly retracted into the lower sheathed portion. Horst's "Rippen-muskeln," which run longitudinally underneath the rows of cones in the proboscis, probably serve to draw in this upper part. Underneath every spike on the proboscis there is a hollow space (fig. 1, *sp.*) in communication with the body cavity, which has also been described of Priapulus bicau-datus (Horst).[1] I presume that the space which is to be found beneath every cone can be rapidly filled with blood, communicating freely with the general body cavity, and in this way the retracted cone may be pushed out. This view is further favoured by the great advantage such a mechanism would have as a protection for these slender structures.

Willemoes-Suhm,[2] who observed living specimens of Pria-pulus, tells us that they are in the habit of burying themselves into the sand by rapidly pushing out the proboscis, and with

[1] Horst, loc. cit., p. 20.
[2] Willemoes-Suhm, "Biologische Beobachtungen über niedere Meeresthiere," 'Zeitschr. f. wiss. Zoologie,' vol. xxi, p. 386.

the same swiftness drawing it in again. Unless the little spikes were retractile I think they would be damaged in this process. In support of this view I may also mention a case where similar organs occur which have been observed in the act of being drawn in. I am alluding to the careful researches of Eisig[1] on the cup-shaped organs of the Capitellidæ. He says, " Die Basis des im übrigen soliden Sinneshügels ist mit einer kleinen Höhlung versehen, welche zunächst von den Wandungen des Hügels, sodann aber von denjenigen des Hautmuskelschlauchs begrenzt, direct in die Perivisceralhöhle übergeht. An die Hügel inseriren sich mehrere Muskeln, deren einer der Retractor, den freien Hügel mehr oder weniger tief einzustülpen vermag. Es ist der Druck des Blutstroms, der sowie den Rüssel und die Tentakel, auch das eingezogene Haarfeld wieder zur Ausstülpung bringt."

The two special sets of muscles in the proboscis which have been mentioned above and which also occur in Halicryptus run underneath the rows of spikes, one on each side, and join the longitudinal muscles of the body wall in the trunk (fig. 11, *r. m.*).

Horst supposes that the above-mentioned hollow spaces are not without importance in the act of respiration. I have just stated my own view on this subject, and need not recur to it again.

As regards the innervation of these organs I have not come to any definite results, although both osmic acid and chloride of gold were used. In some cases, indeed, I saw something like nerve-fibres at the base of a spike, but I was not able to positively prove that what I saw were really such.

Saenger is quite determinate in his assertion that rings of nerves surround the body, one in every segment, similar to those discovered in Sipunculus nudus. In pl. x, fig. 16, he has a drawing representing a lateral nerve going off from the nerve-cord, but as in his diagram the latter is separate from the hypodermis instead of lying inside it as a modifi-

[1] Eisig, " Die Seitenorgane und becherförmigen Organe der Capitelliden," ' Mitth. aus d. Zool. Station Neapel,' vol. i, p. 280.

cation of hypodermic cells, I do not attach much importance to this. Hence I must still regard the view that these dermal spikes are supplied with special nerves as doubtful. A further examination to clear this is much needed.

Ehlers,[1] who was the first to describe the little cones on the proboscis, saw quite correctly that the hypodermic layer was continued into their interior, but he believed that they were hollow and that the hollow cavities might possibly communicate with the perivisceral space. Graber,[2] on the other hand, mistook the hypodermis for a part of the circular muscles of the proboscis, and describes the spikes as being filled up with their prolongations. In their description of Priapulus bicaudatus Daniellsen and Koren[3] agree with Ehlers in looking upon the spikes as hollow outgrowths invested with an internal lining of epithelium. I will give the following passage in their own words :—" In the hollow of every spike, at the base, is seen an almost round, comparatively large gland (figs. 4, e.; 5, e.) composed of connective tissue, covered internally with round cells (figs. 4, f.; 5, f.), and from the arcuate portion of these issues the excretory canal (fig. 4, g.) which, passing up through the hollow of the spike (figs. 4, h.; 5, g.), disembogues exactly where the epithelial integument of the latter terminates (figs. 4, i.; 5, h.). The gland contained a viscid, granulous, pellucid substance, which we observed once or twice in the aperture at the free extremity of the spikes."

There is no doubt that these authors have seen Horst's " Integumentalhöhlen " with their containing blood-corpuscles, which are the glands they mention. Neither Horst nor myself have seen anything of an excretory canal.

Although Saenger's figures leave very much to desire, his investigations seem to have been made with great care, and it is unfortunate that his works, being written in Russian, are practically inaccessible. He states on p. 212 of the work cited before, that the interior of the spikes contains hypodermic

[1] Ehlers, loc. cit., p. 224.
[2] Graber, loc. cit., p. 62.
[3] Daniellsen and Koren, ' Den Norske Nordhavs Expedition,' p. 14.

cells, and he even observed the slender hairs projecting from
the apex of the tubes which I described. This latter fact
escaped the notice of all other observers.

Horst's[1] statements differ from my own in some respects,
in so far as he holds that the interior of the spikes consists
of irregularly branching cells of the hypodermis. The cells
are said to converge towards their base and to have a fibrous
structure, which gives them the appearance of radiating from
the cutis lying underneath (see also his pl. ii, figs. 1, 2, 4, s.).

The spikes of the body, of which we have seen that they are
arranged in parallel rings round the body, are comparatively
few in number. On account of the great contraction of the
circular and longitudinal muscles of the body, these minute
organs almost disappear within their folds or become so con-
torted that they are of no avail for the study of their
histological details. They can only be profitably studied with
good immersion systems, but on account of the above-mentioned
disadvantages I was only able to obtain one or two of them
which were at all satisfactory. Their structure, although on
the main points agreeing with the spikes on the proboscis,
shows a few points of difference, and it seems to have reached
a higher state of development. The whole organ has the form
of a conical or somewhat cylindrical elevation. The cuticula
(fig. 2, c.) is thick and slightly sunk in at the apex of the cone,
leaving an aperture at the centre. The outer hypodermic
cells become again elongated just as in the proboscis, but now
we come to the main difference. The cells in the axis of the
cone are enlarged at their upper part and assume a club-shaped
form, their bases becoming apparently resolved into a net-
work of fibres (fig. 2, c. c.). Perhaps the whole organ may be
constructed on a similar plan to those of Halicryptus, which
will be described below. Each of these central cells bears a
short stiff hair ending freely into the surrounding medium, and
as far as I could ascertain without the intervention of a special
membrane as was the case in the proboscis. This, however, is
nevertheless very probably present, and may have only been

[1] Horst, loc. cit., p. 18.

destroyed in cutting the section or by the action of the alcohol.

Ehlers[1] does not give us much information as to the spikes on the body proper, and merely states that they are small cylindrical elevations truncated at their upper extremity. "Their height is 0·143 mm. and their diameter 0·111 mm., and in their interior, chiefly at the base, lies the substance of the subcuticular layer." The only other observer in whose writings I can find anything about these spikes is Horst.[2] He makes mention of the fact that the hypodermic cells form a continuous layer on the internal surface of the cuticle. The central part is said to contain a network of nucleated fibres.

We now come to the consideration of those dermal organs about which there prevails a good deal of difference of opinion among the various writers. I am alluding to the papillose clusters of dermal processes at the posterior part of the body (fig. 4, *p.*) Even with a strong lens nothing but pretty considerable thickenings of the nature of warts can be made out. On examining the cuticle separately, however, groups of elevations can easily be seen, in the centre of which a pore is to be found (fig. 9). The cuticle here appears to sink into small funnel-shaped pits. In reality, however, these pits are a number of extremely minute tubes, only visible under very high power. In a surface view their real nature might quite easily be mistaken, and it is probably this reason which induced Ehlers to describe them as pits. The general arrangement as seen by a low power is shown in Ehlers' monograph on the genus Priapulus (pl. xxi, fig. 18) (see also my diagram, fig. 4). The main structure of the warts on which the small tubules stand is made up of modified hypodermic cells. These are elongated and filled with granulated contents (fig. 10). The cells differ considerably from those of any other part of the body in being packed closely together and in being of a much greater width. Figure 5 is an attempt to make these statements clearer by showing one of these organs in a longitudinal

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[1] Ehlers, loc. cit., p. 225.
[2] Horst, loc. cit., p. 18.

section.   At the apex of the tubules I noticed a little pit (*p.*) into which opens a very delicate canal (*o.*) from a flask-shaped portion (*f.*) below, which communicates again with several large cells (*h.*).

These details do not appear in objects coloured with carmine, and I therefore tried one of the aniline dyes, viz. methyl violet, which has been successfully used for a similar purpose by Spengel[1] in order to demonstrate the excretory ducts of glandular organs in the skin of Echiurus Pallasii.   The aniline dyes are said to have the property of staining secretions very markedly.   If this be the case I think I am quite justified in regarding these organs as organs of secretion, the cells forming the warts having been stained of a deep blue together with the flask-shaped portion (fig. 5, *f.*) and the duct, while the other hypodermic cells only assumed a very slight bluish tinge. Other investigators maintain, on the contrary, that aniline dyes cannot be trusted and that their action is very fickle.   Nevertheless another circumstance besides the aniline method seems to give the above-stated view an additional support.   In the spirit specimens the immediate surroundings of the warts were thickly covered with a yellowish sticky material, which had to be brushed off in order to allow of a more accurate scrutiny.

The supposition that these organs might be of a secretory nature has only been put forward by Ehlers, while Saenger maintains that there are no pores at all.   Graber[2] believes that the investigations of Ehlers are in want of improvement, and scorns at the idea of the existence of pores.   These "secretory organs"—if I may venture to call them such—do not exist in either Priapulus bicaudatus or Halicryptus spinulosus. Horst as well as Daniellsen and Koren do not mention anything about them with regard to the former and I have not found them in the latter.

[1] Spengel, "Beiträge zur Kenntniss der Gephyreen," 'Zeitschr. f. wiss. Zoologie,' vol. xxxiv, p. 464.

[2] Graber. loc. cit., p. 63.

Halicryptus spinulosus.

As far as I have been able to ascertain, the spikes on the proboscis agree with those of Priapulus. The cuticula, however, surrounding the spikes, differs somewhat in not forming a sheath round them, but a crest of little lancet-shaped blades. Figure 11 represents a somewhat diagrammatic view of their general arrangement.

At the line of junction between the proboscis and the body proper (fig. 11, *j.*) the spikes become curiously modified. They are not always so regularly formed as I have indicated them on the diagram, and generally there are three circular rows of them surrounding the proboscis. Occasionally we find some that do not exhibit those two prongs at the apex; in fact, there may be a number of transitory stages between the ordinary form of the proboscis and this peculiarly modified form.

According to Saenger we find at the apex of the subcuticular elevation a large transparent cell containing a quantity of yellowish droplets looking like fat. He observed the animal in the living state, and noticed that under ordinary circumstances these little prongs are never withdrawn into the interior. They form the boundary up to which the invagination of the proboscis takes place, and probably act as a kind of support. By means of these prongs the worm keeps his position even when the anterior part is drawn in, and they may also be advantageous in locomotion. I have not been able to investigate their histological structure.

The most interesting of the dermal organs of Halicryptus are those of the body proper. They are almost twice as long as the ordinary spikes of the proboscis (fig. 3 A, and fig. 11, *sp. t.*). We can distinguish two parts, a lower and an upper. The former rests on a broad base tapering somewhat towards the apex, while the latter consists of a slender portion resembling in external appearance the sting of a bee. The hypodermic cells filling up the interior of the lower portion are modified into three different sets, one being replaced by another as we

approach the axis of the structure.   This is best understood by examining a longitudinal section such as fig. 3 A.   The hypodermic cells forming the circumference of the spike elongate just as we have seen it before in Priapulus.   Internally to these we now find large pear-shaped cells (figs. 3 A, 3 B, *p. c.*), containing a protoplasmic network and nucleus, and tapering above into a fine filament which suddenly swells up again into a club-shaped portion.   This set of cells again surrounds another set (figs. 3 A, 3 B, *i. c.*), which I have not been able to trace clearly, but which are probably filamentous in shape from base to apex and end in long hairs.   The latter (*l. h.*) occupy the hollow interior of the sting.   A membrane (fig. 3 A, *m.*) through which the hairs project stretches across the opening from the lower into the upper part of the organ.   A cross-section a little lower down (fig. 3 B) stained with chloride of gold, exhibits internally a cluster of small dark-stained cells (*i. c.*) surrounded by larger lighter ones (*p. c.*), the latter representing the upper parts of the pear-shaped cells (I may mention that in sections stained with carmine according to Grenacher's prescription I have likewise obtained very instructive views of the minute structure of these organs).   The cuticle encircling the two groups of cells in this part is made up of an internal and an external dense part, staining darker than the rest, which lies between.   Further down towards the base it assumes its ordinary composition again, the internal dark portion being wanting.   Fig. 3 represents a cross-section through the sting, showing the hairs originating from the central group of cells and surrounded by the cuticular wall.

Having considered the anatomical and histological details of all these sensory organs, it now remains, after having made a few remarks on similar organs in other animals, to show their relation to these.

In Sipunculus nudus, which has recently been reinvestigated by Andreae,[1] structures composed of modified hypodermic cells have been discovered, the bases of which are in connection with nerves (pl. xii, fig. 9).   No sensory hairs

[1] Andreae, loc. cit., p. 219.

were observed, which may be due to their having been destroyed through the action of the alcohol.

Keferstein[1] makes mention of similar organs in Phascolosoma, but he seems as yet doubtful whether he should call them "sensory organs," not having been able to find any connection with nerves. Soon after, however, he published another paper[2] in which he calls them organs of touch (Tastorgane), and proves that they are supplied by special nerves.

One of the more recent writers is Teuscher,[3] who, in his description of the skin of Phascolosoma, mentions utricular bodies in connection with two or three nerves-fibres. "Their interior contains, in a finely granulated mass, a number of larger grains which seem partly attached to threads hanging down from the apical part." This passage reminds me very much of the elongated hypodermic cells which I have so often described, and it seems to me not at all unlikely that the above-mentioned long threads are nothing but similar cells with their conspicuous nuclei. Greeff,[4] who made the Gephyrea armata his special study, gives us much valuable information as to the occurrence of organs of touch (Tastpapillen), which, however, lie in a layer of connective tissue beneath the epithelial or hypodermic layer. They are either arranged in rings round the body, or they may be spread all over. The nervous connection was traced all the way from the nerve-cord to these papillæ.

I have already had the opportunity in another place of referring to Eisig's researches "über die Seitenorgane der Capitelliden."[5] He describes sensory organs having the form of buds, and others sunk down into cup-shaped invaginations of

[1] Keferstein, "Untersuchungen über niedere Seethiere," 'Zeitschr. f. wiss. Zoologie,' vol. xii, pp. 41, 42.

[2] Keferstein, "Beiträge zur Anatomie und systematischen Kenntniss der Sipunculiden," 'Zeitschr. f. wiss. Zoologie,' vol. xv, p. 405.

[3] Teuscher, "Notiz über Sipunculus und Phascolosoma," 'Jenaische Zeitschrift,' vol. viii, p. 495.

[4] Greeff, "Die Echiuren," 'Nova Acta Akad.,' vol. xli, p. 44.

[5] Eisig, loc. cit., p. 280.

the skin, both of them having sensory hairs. He has established experimentally that the apparently different structures are one and the same thing, the latter being simply an invagination of the first kind.

The most noteworthy analogy to the sensory organs of Priapulus and Halicryptus, however, is to be found among the lower Vertebrates, such as fishes and larval Amphibians. In order to show how closely the organs of which the so-called lateral line in fishes is composed of agrees with those I have described, I will give a few extracts from some of the more important works on this subject.

Leydig[1] was the first to prove the nervous nature of these organs, which were generally believed to excrete mucus, and showed that they were sensory organs peculiar to fishes. A few years later he wrote an excellent and well-known treatise[2] on sensory organs in general, and attributed to those found in the lateral line of fishes the function of a sixth sense unrepresented in the higher Vertebrates.

M. Schultze, as well as F. E. Schulze, have extended the knowledge about these organs very considerably. It was the latter who first discovered the peculiar protuberant structures in the skin of young fishes, and indicated that they were peripheral sense organs corresponding to those of the lateral line found in adult fishes. Shortly after he published another paper[3] in which he describes their histological structure :—
" From the epithelial cells, of which these organs are chiefly made up and which stand in connection with nerves, I saw a number of delicate stiff hairs projecting into the water similar to those found on the Crista acustica, only much shorter." Moreover, he describes " a slender tube, rising from the margin of this structure, open at the end and obliquely truncated."

[1] Leydig, " Ueber die Schleimkanale der Knochenfische," 'Müller's Archiv,' 1850.

[2] Leydig, " Ueber Organe eines sechsten Sinnes," 'Nova Acta Leop. Carol.,' vol. xxxiv.

[3] F. E. Schulze, " Ueber die Sinnesorgane der Seitenlinie bei Fischen und Amphibien," 'Archiv f. mikrosc. Anatomie,' vol. vi, p. 63.

The latter evidently acts as a sort of protection to the fine hairs, and is analogous to those of Priapulus and Halicryptus.

Langerhans[1] and also Solger[2] give further details as to their composition. The latter says : "Two parts may be readily distinguished in longitudinal sections—firstly, an outer integument, and secondly, an inner nucleus. The former is constructed of several layers of long cylindrical cells. These mantle-cells surround the second inner part which form a group of club or pear-shaped cells (Kolben oder birnförmig gestalteten Zellen) (see Langerhans, pl. xxxi, figs. 10, 11, 12). The latter correspond to Bugnion's[3] "cellules pyriformes" and to the pear-shaped cells in Halicryptus. Bugnion's "cellules-à-bâtonnet" would then be analogous to an internal group of cells I described (see also Bugnion, pl. xiii, figs. 1, 3, 6). Moreover, Merkel,[3] in his very detailed description of the sensory organs of Vertebrates, points out that the set of cells surrounding the hair-cells secretes a sieve-like "membrana limitans," through the meshes of which the hairs project into the surrounding medium. A similar limiting membrane was described above with regard to Priapulus and Halicryptus (fig. 1 and fig. 3 A, m.). This furnishes us with an additional point of similarity between the sensory organs of these two Gephyreans and the corresponding structures of the lower Vertebrata.

The Nervous System.

The results I obtained as regards the nervous system agree in general with those of Horst. It is composed of a ventral

[1] Langerhans, "Ueber die Haut der Larve von Salamandra maculosa," 'Archiv f. Mikrosc. Anatomie,' vol. ix.

[2] B. Solger, "Zur Kenntniss der Seitenorgane der Knochenfische," 'Centralblatt d. medic. Wiss.,' 1877, p. 818.

[3] E. Bugnion, "Sur les organes sensitifs," 'Bulletin de la Société Vaudoise des Sc. nat.,' vol. xii, p. 268. (Dissertation inaugurale.)

[4] Merkel, 'Ueber die Endigungen der sensiblen Nerven in d. Haut d. Wirbelthiere,' Rostock, 1880.

cord and an œsophageal ring. Both in Priapulus and Halicryptus the nervous system lies entirely in the ectoderm—a condition which is of rare occurrence, but which has, as far as I know, been likewise noticed in a few Annelids—for example, Hesione and Ovenia.

On the body proper the position of the cord is well-marked externally by a shallow groove running along the ventral surface, whose two sides are slightly raised, while two of the spike-bearing ribs indicate its continuation on the proboscis. The nerve-cord is not continued into the tail appendage (Schwanz-anhang), but ends at the posterior part of the body in a considerable swelling. Anteriorly it divides into two branches which surround the œsophagus. Its position is here again indicated externally by a very deep groove (fig. 8, *g.*).

Although it appears in cross-sections as if swellings existed in the cord at regular intervals, I believe this to be merely due to the powerful contractions of the annular muscles, allowing the cord to bulge out slightly in the intervening spaces. In the œsophageal ring, however, a real thickening, already observed by Saenger, exists dorsally. As regards the size of the nerve-cord in the ring as compared with that in the body, the diameter in the former is about two to three times as great.

All previous observers state that the nervous system lies immediately under the hypodermis, between it and the annular muscles. In reality, however, it is placed within the hypodermis, the ganglionic cells being simply modified hypodermic cells and the fibrils their processes. As the hypodermis approaches the cord its cells become elongated just as we have seen before in the case of the spikes, and ultimately they swell up, becoming modified into ganglionic cells (figs. 6, 7, 8). Internally the cells of the hypodermis send out numerous processes. These are well seen in the long cells close to the large mass of nerve-fibres in the body (fig. 6, *h. p.*). The dorsal part of the cord is wholly taken up by the nerve-fibres (figs. 6, 7, 8, *f.*), and on each side ventrally we find a cluster of ganglionic cells (fig. 6, *g. c.*). A similar arrangement has been

observed in Priapulus bicaudatus and Hamingia,[1] also in Sternaspis,[2] which, however, has now been removed from the Gephyrea. In Echiurus the cellular part of the nervous system is also arranged in form of two peripheral rows of cells.

Immediately above the two masses of nerve-cells on each side of the cord the space seems filled up with connective tissue (fig. 6, *h. p.*) which would correspond to the great development of the same in Priapulus bicaudatus (see Horst, fig. 15). Whether this is really connective tissue or whether the branches of the hypodermic cells assume a structure simulating connective tissue, I have not been able to settle. In the peripheral part of the central mass of nerve-fibres a few cells may be seen scattered here and there (figs. 6, 7, *n. c.*) In the proboscis two longitudinal muscles appear on each side of the cord externally to the annular muscles (fig. 7, *m.*). The result is that the cord becomes compressed laterally and the two clusters of cells become fused into one (fig. 7, *g. c.*)

A section through the nerve ring is shown in fig. 8. The ring, as we have seen before, is situated at the base of a groove (*g*), surrounding the mouth. The retractor muscles of the proboscis are attached to the ring, while it is itself again closely united to the skin by muscular tissue. The greater part of the nerve ring is taken up by fibres (*f.*) and ordinary cells (*g. c.*), and the latter send their processes into the fibrous portion. A few larger cells lie above the fibrous part internally (fig. 8, *g, g.*)

The considerable swelling at the posterior end of the ventral nerve-cord has been very ably described by Horst with regard to Priapulus bicaudatus. I can only confirm his statements in the most essential points. His figure 14 is a very good representation of a cross section. The hypodermic cells send their branches to the interior from the peripheral part, while the central portion is taken up by smaller ganglionic cells, which are surrounded by larger ones. Such is the arrange-

[1] Daniellsen and Koren, loc. cit., p. 30.

[2] Sluiter, " Ueber einen indischen Sternaspis," 'Naturkundig Tijdschrift vor Nederl. Indië,' vol. xli, p. 274.

ment in the posterior part of the ganglion. A little more towards the anterior end nerve-fibres make their appearance in the centre between the small cells and gradually displace the cellular part, until they occupy the position which I have described in dealing with the arrangement in the body proper.

In his anatomy of Halicryptus, Saenger describes lateral nerves going off from the main trunk and surrounding the body in a similar way as in Sipunculus nudus. The median fibrous mass, he says, remains without a change at the points where the branches originate, and does not send any fibres into them. In spite of my endeavours to find these lateral nerves, I have not been able to identify them. Horst did not find them either. On the other hand, Daniellsen and Koren[1] mention that the central nervous cord sent off numerous branches to the skin and muscles.

In Echiurus Pallasii[2] the cellular portion runs along the whole of the cord in its peripheral part. A canal situated immediately under the dorsal median line is mentioned in Greeff's[3] description of Echiurus, who supposes it to be a remainder of the invagination from the Ectoderm. According to Andreae[4] the arrangement in Sipunculus nudus is rather different. The cord also lies internally to the muscular system, and consists of a sheath of connective tissue forming an external neurilemma. The nervous elements are surrounded by a similar internal sheath, and between the two lies a finely-granulated mass with small nuclei but without cells.

It will be seen by the above description that I have not been able to trace any peripheral nerves coming off from the nerve-cord. It would be rash to assert on the strength of this that they do not exist, and a further examination of fresh specimens is needed to clear up these doubts. At the same time I think it quite possible that the whole of the hypodermis acts as a kind of nervous layer. On the other hand, the well-

[1] Daniellsen and Koren, loc. cit., p. 17.
[2] Spengel, loc. cit., pp.484—86.
[3] Greeff, loc. cit., p. 85.
[4] Loc. cit., p. 249.

developed sensory organs, as well as the organisation of the nerve-cord, seem to lead to a different conclusion, and I hope these points may soon be definitely settled.

EXPLANATION OF PLATE XIV,

Illustrating Mr. Robert Scharff's paper " On the Skin and Nervous System of Priapulus and Halicryptus."

Fig. 1. Priapulus.—Longitudinal section of a spike on the proboscis. *h.* Hypodermis. *ci.* Inner layer of cuticula. *ce.* Outer layer of cuticula. *m.* Limiting membrane. *sp.* Hollow space underneath spike in communication with body cavity.

Fig. 2. Priapulus.—Longitudinal section of a spike on the body proper. *h.* Hypodermis. *ci.* Inner, *ce.* outer layer of cuticula. *c. c.* Club-shaped cells of the hypodermis bearing short hairs.

Fig. 3 A. Halicryptus.—Longitudinal section of a spike on the body proper. *h.* Hypodermis. *ce.* Outer, *ci.* inner layer of cuticula. *e. c.* External modified hypodermic cells. *p. c.* Pear-shaped cells. *i. c.* Internal modified hypodermic cells. *m.* Limiting membrane. *l. h.* Long hairs projecting through membrane.

Fig. 3 B.—Cross section below the limiting membrane. Letters same as above.

Fig. 3 C.—Cross section of the upper portion of spike, showing it to be a tube containing hairs which seem to radiate outward.

Fig. 4. Priapulus.—Posterior part of the body. A ventral view, showing nerve-cord (*n.*), papillæ (*p.*), also the tail-appendage (*t.*).

Fig. 5. Priapulus.—Diagrammatic longitudinal section of part of a papilla. *c.* Cuticle. *h.* Hypodermic cells, greatly elongated and ending above in a flask-shaped portion (*f.*) with an opening (*o.*).

Fig. 6. Priapulus.—Cross section of nerve-cord in the body. *c.* Cuticula. *h.* Hypodermis. *a. m.* Annular muscles. *f.* Fibrous nerve-mass. *g. c.* Ganglionic cells. *h. p.* Processes of hypodermic cells. *n. c.* Scattered cells.

Fig. 7. Priapulus.—Cross section of nerve-cord in the proboscis. *c.* Cuticula. *h.* Hypodermis. *f.* Mass of nerve-fibres. *g. c.* Ganglionic cells. *m.* External longitudinal muscles of the proboscis. *g. c.* Ganglionic cells.

Fig. 8. Priapulus.—Cross section of œsophageal ring. *h.* Hypodermis. *g.* Groove surrounding oral aperture. *d.* Cutis. *f.* Mass of nerve-fibres. *g. c.* Ganglionic cells. *g. g.* Larger ganglionic cells. *r. m.* Retractor muscles of the proboscis.

Fig. 9. Priapulus.—Surface view of a cluster of papillæ at posterior end of body.

Fig. 10. Priapulus.—Cross section of a papilla, showing the cells filled with granulated contents.

Fig. 11. Halicryptus.—Strip of skin from the junction between proboscis and body, exhibiting three different kinds of spikes. *sp. p.* Spike of proboscis. *sp. t.* Spike of body. *j.* Line of junction between proboscis and body proper, with the peculiar forked spikes. *r. m.* External longitudinal muscles of proboscis. *l. m.* Internal longitudinal muscles. *c. m.* Circular muscles. *sp.* Hollow space underneath every spike in communication with perivisceral cavity.

Fig. 12.—General view of Priapulus caudatus, three times natural size.

Fig. 1.

Fig. 2.
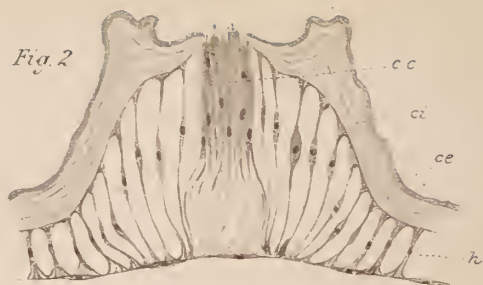
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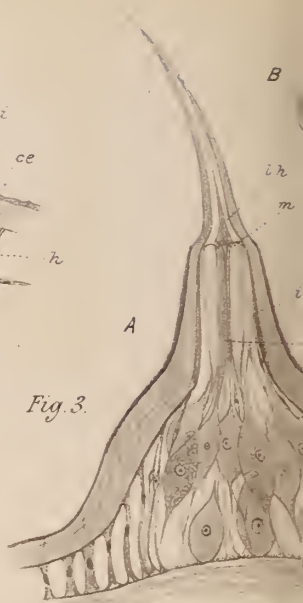
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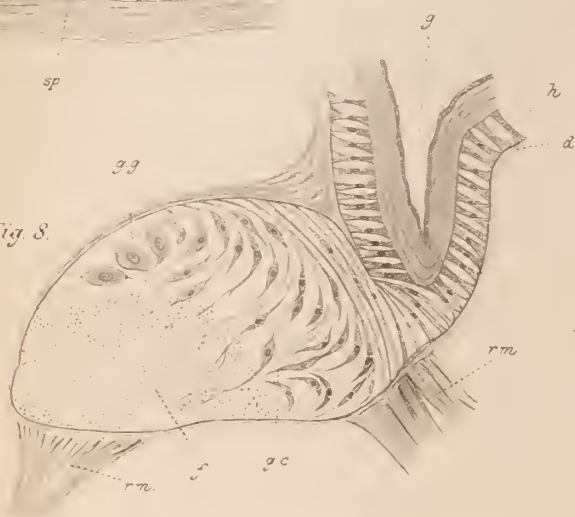
Fig. 7.
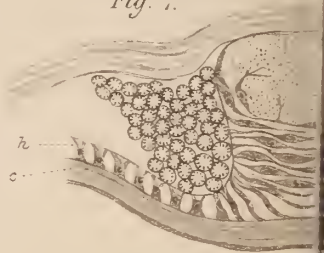
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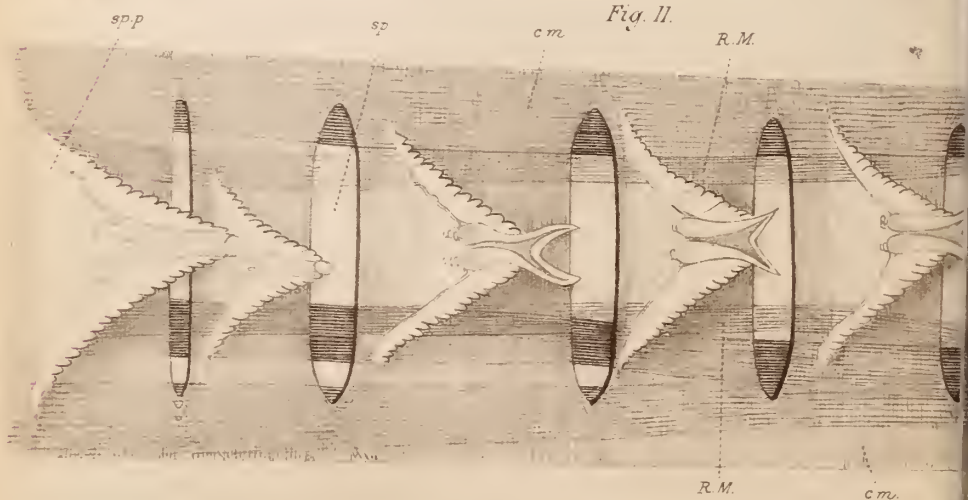
Fig. 4.

Fig. 5.

Fig. 6.
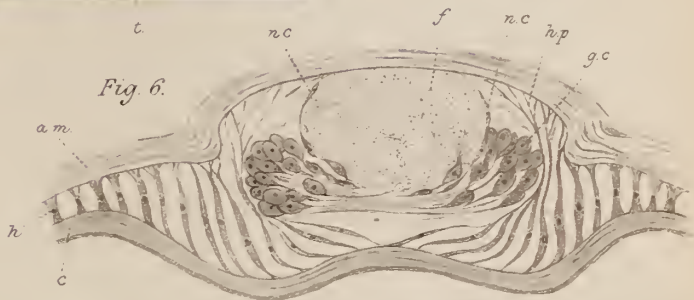
Fig. 9.
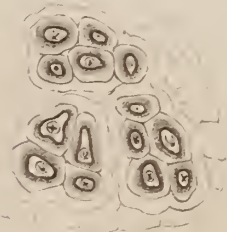
Fig. 10.

Fig. 12.
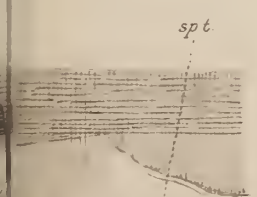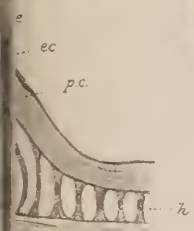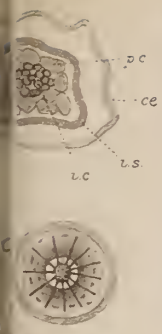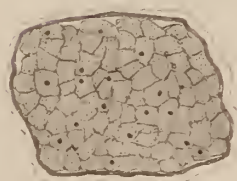
The Eye and Optic Tract of Insects.

By

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With Plates XV, XVI and XVII.

THE structure of the eyes of the Arthropoda has been a favourite subject with morphologists for many years, and many beautiful monographs have been published detailing the results of the careful observations that have been made upon this subject. Until the last few years the observations stopped short at the basilar membrane, and the tract lying between the percipient elements and the brain remained unknown. Of late years, however, several papers have appeared dealing with the optic tract and its relations to the eye proper, or ommateum (Lankester and Bourne), and as some of these have attempted to throw doubts upon what were formerly considered to be well-founded homologies, I have gathered together in this memoir some of the most interesting results of the researches I have been carrying on for the last two or three years with the hope of being able thereby to throw some light upon these obscure or disputed points. In order to make my memoir more complete I have carefully revised the structure of the ommateum itself in Musca vomitoria, and I shall here give a detailed account of it based upon my own observations before I proceed to describe the nervous elements which connect it with the brain.

The descriptions which have been published of the ommateum of insects in general and Musca in particular, are now so

numerous that to attempt to refer to previous authors in the course of my description would only tend to hamper and obscure it. Consequently, I shall leave all reference to previous authorities to a separate section at the end of the paper, when I shall attempt to discuss the various disputed points and clear up the inconsistencies between my own descriptions and those of other observers.

§ 1. The Eye and Optic Tract of Musca vomitoria.

The eyes of the blow-fly are large brown protruding structures situated upon the anterior surface of the head. Externally they are protected by the chitinous corneæ which are broken up into a large number of biconvex facets. The number of these facets varies in the individual. Müller (17) gives 4000 for Musca domestica. In a vertical section through the middle of an eye of a blow-fly which had emerged from its pupa twenty-four hours, I was able to count 62 (Pl. XV, fig. 2). In some I could count as many as 80, and in some only 40. The external convexity of the facet is usually formed of a greater arc of a sphere than the internal, so that between each facet there may be seen, internally only, a small flat surface. This is invariably covered with a dense pigment. This difference between the external and internal convexity of the corneal facets gives a plano-convex appearance to sections that do not pass through their centres (v. Pl. XV, fig. 3, c).

Internally to the cornea is situated the so-called pseudocone, which is ensheathed by two or three nucleated pigment-cells (fig. 3, $pg._1$).

The pseudocone consists of four cells, each of which consists of a clear transparent external portion and a smaller protoplasmic portion containing the nucleus situated internally ($n.p.c$). The clear transparent portion of each pseudocone-cell contains in the living eye a watery or perhaps slightly albuminous fluid, for in specimens preserved in spirit all that can be seen of this portion of the cell is a band of protoplasmic substance stretching from the nucleus to the cornea, and staining deeply with hæmatoxylon. Between each element of

the ommateum or ommatidium as I shall call it, adopting the term introduced by Carriere (3) there is situated in the region of the base of the pseudocone, a large pigment-cell (figs. 3 and 10, $pg._2$). Each of these pigment-cells consists of a central rounded portion containing a large spherical nucleus and two or more delicate processes which pass externally (ex.) to the flat portions of the internal surface of the cornea, and internally (in.) to accompany the retinulæ towards the basilar membrane.

The rhabdom of Musca consists of a bundle of six long delicate chitinous rods, more or less firmly united together (fig. 3, rh.). The rhabdomeres or elements of the rhabdom are more clearly distinguished from one another in the outer part of their course (fig. 4) than they are in the inner part of it (fig. 5.) The rhabdom is surrounded by the retinulæ. These are six in number, and in the outer region of their course are free from one another (fig. 4, r.), but in the inner region are fused into a sheath.

Each retinula element possesses a nucleus just behind the nuclei of the pseudocone (fig. 3, nr.) and some of them possess an additional nucleus in the middle of their course (fig. 3, nr_1.). Thus we find a ring of six retinular nuclei around the central rhabdom, just behind the pseudocone and two or three nuclei somewhat more irregular in position about half way down.

When quite fresh the retinulæ are of a deep carmine colour, but this soon fades away under the influence of the light, and the retinulæ are left with a yellowish-brown colour. Between the ommatidia internally there are found pigment-cells (figs. 3, $pg._3$), each of which stands on the basilar membrane and sends a fine process outwards towards the internal process of the external pigment-cell ($pg._2$). The pigment-cells are filled with bright carmine-coloured granules which change to a deep brown colour when treated with alcohol.

The basilar membrane of Musca is very thin and perforated for the passage of tracheal diverticula and the optic nerve-fibrils.

Between the ommatidia are situated long tubular thin-walled air-sacs, which may be traced into connection with the nume-

rous branching tracheæ which traverse the nervous network just behind the basilar membrane. These tracheal vesicles are easily seen in fresh-teased specimens of the eye of Musca, but they are not easy to see in thin sections through hardened specimens, as their walls are very thin, unpigmented, and stained with difficulty.

Turning now to the anatomy and histology of the optic tract. Between the brain and the basilar membrane of the eye we are able to distinguish three distinct ganglionic swellings. The first one of these (fig. 1, *op.*) is separated from the cerebral by a narrow constriction, which, as Berger (2) has pointed out, is the homologue of the optic nerve of the other Arthropoda. I shall call it in this paper the opticon.

The second ganglionic swelling is separated from the opticon by a tract of fine nerve-fibrils, which partially decussate, and a few scattered nerve-cells; and I shall call it the epi-opticon (fig. 1, *e. op.*).

The third ganglionic swelling is much flatter in shape than the others, is separated from them by a bundle of long optic nerve-fibrils, which cross one another; and I shall call this the peri-opticon (fig. 1, *p. op.*).

The three optic ganglia, together with the cerebral ganglia, are surrounded by a sheath of very densely-packed nerve-cells (fig. 1, *n. c. s.*), the " Punktsubstanz" of Leydig. I have examined this sheath very carefully in numerous types, and I find it to consist of densely-packed cells, each composed of a very large nucleus surrounded by a very delicate envelope of cell-protoplasm. The individual cells are connected with one another by protoplasmic connectives, and in some places fine nerve-fibrils anastomose between the cells, and are probably connected with them by fine anastomosing branches.

In the silkworm moth (Pl. XV, fig. 15) the protoplasmic sheath surrounding each nucleus is thicker than it is in Musca and most other Arthropods, so that the cellular nature of this sheath is in this case very clearly seen.

In the brain of the developing bee the cells are not so densely packed as they are in adult animals, so that in well-

stained specimens it is easy to distinguish the cell-protoplasm and the anastomosing branches (Pl. XV, fig. 6).

I have spoken of these cells as nerve-cells because it is necessary to distinguish them from ganglion-cells, which are more isolated in position, and possess a considerable quantity of cell-protoplasm. I shall refer to this point again in Section 3.

The opticon itself consists of a very fine granular matrix, traversed throughout by a fine meshwork of minute fibrillæ, similar to the minute network of primitive fibrillæ described by Gerlach in the mammalian brain and spinal cord. This description of the minute structure of the opticon applies equally to the epi-opticon and principal ganglia of the body. As this tissue is very commonly met with in the animal kingdom, and has not, as far as I am aware, yet received any separate name, I propose to call it a neurospongium. In many insects the neurospongium of the opticon is traversed by fibrils, and in some cases it contains a few scattered nerve- or even ganglion-cells.

The epi-opticon is connected with the peri-opticon by a bundle of decussating nerve-fibrils, and as these fibrils approach the peri-opticon they are connected with a number of scattered nerve-cells (fig. 2, *n. c.*), and at the decussation two or three larger nerve-cells may be seen in each section (fig. 2, *gs.*)

The peri-opticon of Musca is composed of a number of cylindrical masses of neurospongium arranged side by side, which I shall refer to as the elements of the peri-opticon (fig. 2, *p. op.*), and between them a single nerve-cell is very frequently seen.

As this ganglion has been recently subjected to considerable investigation I have taken considerable pains to study it thoroughly. In addition to numerous sections of hardened specimens, made both transversely and horizontally, I have succeeded in isolating the peri-opticons of fresh flies, and teasing them carefully with needles, both before and after staining in gold and other reagents, and the following account is based on these investigations.

The nerve-fibrils coming from the epi-opticon divide into two

or three main fibrillæ on entering the cylindrical elements of the peri-opticon (Pl. XVI, figs. 16 and 17, *Nf.*), and these fibrillæ again subdivide and form the ultimate fibrillar mesh-work of the neurospongium.

Each little cylindrical mass of the peri-opticon is not entirely separated from its neighbours, but is connected with them, either directly by fine fibrillæ, or indirectly by the intermedia-tion of nerve-cells (figs. 16 and 17, *n. c.*).   I have observed also that some fibrils do not break up to form any neurospongium, but pass straight through without undergoing any subdivision (fig. 16, *f.*).

Sometimes the structure of the elements of the peri-opticon is complicated by the deposition of pigment.   This deposition of pigment may take the form either of small cylindrical rods or of a fluted hollow cylinder, or of a smooth hollow cylinder (figs. 8, *a, b, c,* and 9).   The presence of pigment in this region is, however, very variable.   The figs. 8 and 9 were drawn from a permanent preparation I have in my possession now, all the varieties (*a, b, c*) being found in one eye, whilst the other eye has no pigment at all in this region.

The number of the elements of the peri-opticon does not seem to bear any relation to the number of ommatidia; in some of my preparations they seem to be of about the same number; in others they are considerably less, and in one of my sections I could only count half as many.

Externally a number of fibrils leave the elements of the peri-opticon, and at once form a complicated anastomosis with the numerous nerve-cells found in this region, which in its turn furnishes the fibrils which pass through the basilar membrane to supply the retinulæ.

In this "terminal anastomosis" are found a number of branching tracheæ—distinguished by their spirally-marked walls—which run more or less parallel with the basilar mem-brane (fig. 2, T.).   They spring from two main tracheal trunks (*T.t.*), situated at the sides of the head behind the eyes.   The tracheæ of the terminal optic anastomosis supply the tracheal vesicles which are found between the ommatidia.

In Musca vomitoria only a few tracheæ with spirally-marked walls are found behind the peri-opticon, and these arise from separate tracheal trunks situated at the sides of the optic tract.

§ 2. The Minute Anatomy of the Optic Tract of various Insects compared.

When the brain and optic tract of a very young Periplaneta is examined, it is found that the optic nerve separating the cerebral ganglion from the opticon is much longer in proportion than it is in the adult blow-fly. The opticon is a well-marked ganglion, and the epi-opticon is small, though distinct.

From the epi-opticon, however, the optic fibres pass straight to the ommateum without passing through any intermediate neurospongium. In fact, the peri-opticon described above in Musca does not exist in the young Periplaneta (fig. 18). The optic fibres leaving the epi-opticon do not decussate, but as they approach the basilar membrane they break up into numerous branches which undergo a loose anastomosis. In this anastomosis a few large nerve-cells may be seen (fig. 18, *n. c.*). In the adult Periplaneta the opticon is very large and separated from the cerebral ganglion by a very considerable optic nerve. The fibres connecting the opticon and the epi-opticon decussate (fig. 19, *d.f.*). The epi-opticon is a smaller hemispherical well-marked ganglion, and the fibres which pass from it to the ommateum partially decussate (*Nf.*). My figure (19) is taken from a preparation of the head of an adult male cockroach with fully-developed wings, so that I am inclined to think that in this genus the optic fibres never completely decussate in this region. The number of fibrils passing from the epi-opticon to the ommateum is relatively much larger than in the young, and the nerve-cells in the meshes of the anastomosis are more numerous. The anastomosis is, perhaps, rather denser and more complicated than it is in the young, but I cannot find anything comparable to the loopings of Nepa to be described immediately, nor anything that could be described as a neurospongium.

In Nepa the optic fibres leave the epi-opticon, and without decussating, at once form a very complicated anastomosis in which there are a number of small nerve-cells. Before entering the ommateum the anastomosis passes through the pores of a membrane situated a little distance behind the basilar membrane. A similar perforated membane in this position has been described by Berger (2) and Ciaccio (4) in Musca, but I have not been able to detect it. In that part of the anastomosis which corresponds in position with the peri-opticon of Musca, some of the principal fibrils have the appearance of broadening or flattening out, and when they are examined with a high power these flattened portions are seen to be made up of a number of minute fibrillæ. Between the principal fibrils run ordinary anastomosing branches, transverse connecting fibrils and fibrils which seem to turn round and run back again to the epi-opticon, and which have a looped appearance in the sections. These looped fibrils are also found in the optic tract of the developing bee.

In Musca, as I have described above, the optic fibrils on leaving the epi-opticon decussate and then break up into small cylindrical masses of neurospongium, which together form what I have called the peri-opticon.

In Agrion bifurcatum (figs. 20, 21), one of our common English dragon-flies, the opticon is small compared with the enormously large epi-opticon (fig. 20, *e. op.*) the fibrils passing between the two do not decussate, but after undergoing a considerable anastomosis with one another pass straight across. The decussation of the fibrils between the epi-opticon and the peri-opticon is complete (fig. 20, *nf.*). The peri-opticon is composed (figs. 20, 21, *p. op.*) of a number of long, slender cylindrical elements, each composed of a delicate neurospongium. Between the elements there are a few connecting fibrillæ, and a number of nerve-cells. The terminal anastomosis is much more complicated here than in Musca, and takes up very much more room, so that the peri-opticon is, comparatively speaking, situated some distance behind the basilar membrane (fig. 20, *ta.*).

The terminal anastomosis of Agrion may be conveniently divided into four regions.   First, the region (1) lying nearest to the peri-opticon in which the nerve-cells are numerous, and the fibrils leaving the peri-opticon form a complicated plexus, the region (2), next to this, in which the fibrils have collected into bundles separated by spaces occupied by very thin-walled tracheæ in which there are no spiral markings, and lymph spaces ; next, the region (3), in which the fibrils form a final plexus, and in which there are again a considerable number of nerve-cells, and, lastly, the region (4), in which the fibrils are again collected into bundles, separated by spaces containing tracheæ, which perforate the basement membrane to supply the retinulæ.

In Noctua, Sphinx, and Acherontia, the three genera of Lepidoptera I have examined, the peri-opticon is composed of the usual cylindrical elements, but they are much longer, thinner, and more tightly packed than usual, so that the whole ganglion has a much more compact and spherical appearance than it has in any of the genera we have hitherto considered.

The peri-opticon is closely connected with the epi-opticon by a thick nerve tract in which the nerve-fibrils completely decussate, but neither in this region nor in the epi-opticon nor in the peri-opticon is there any trace of tracheal vessels perforating the tissues.

The terminal anastomosis of the Lepidoptera is most extraordinarily complex, and the four regions described above in Agrion can be readily distinguished (*conf.* Leydig, Taf. x, fig. 2).   In region 2, the large thin-walled, but still spirally-marked tracheæ may be readily seen branching between the nerve-fibril bundles.

The terminal anastomosis of the Lepidoptera is usually very deeply pigmented.   In the bee and the wasp, the only two members of the order Hymenoptera I have examined, the peri-opticon is very similar to that of the Lepidoptera, the elements being long, delicate, and very close to one another. The terminal anastomosis is not so complicated, nor is it usually so densely pigmented.   No spirally-marked tracheæ penetrate the optic tract at any part of its course in Hymenoptera.

In Aeschna grandis, one of the Libellulidæ, owing to the very large size of the eyes, the peri-opticon can be easily seen, on making a simple dissection, to be a large flat ganglion under-lying the basilar membrane. On making a fine section through it it will be seen to differ from that of the forms hitherto described, in that it can no longer be divided into a number of cylindrical elements well marked off from one another, and easily separated by teasing (fig. 7, *p. op.*). In other words, the elements of which the peri-opticon is composed in Musca are here fused into a single mass of neurospongium, which differs from the neurospongium of the epi-opticon and opticon itself only in the fact that it contains a number of irregular spaces (*s.*), which correspond, perhaps, with the spaces between the elements in Musca.

The nerve-cells (*n. c.*), which in the forms hitherto de-scribed are situated between the elements of the peri-opticon, are here found to be scattered irregularly through its sub-stance.

The terminal anastomosis of Aeschna is not so clearly divi-sible into four regions as it is in Agrion, but still the fibrils, on leaving the peri-opticon, may be seen to form a loose plexus with the numerous nerve-cells found in this region (fig. 7, 1), then to collect together into a number of bundles separated from one another by considerable spaces, (2) then to form a second plexus (3) before finally breaking up into the individual bundles (4) which run through the basilar membrane to supply the retinulæ.

In Eristalis lupinus the opticon and epi-opticon are very similar to those of Musca, but the peri-opticon and the neigh-bouring parts present some interesting features (fig. 23). The nerve-fibrils leaving the epi-opticon completely decussate, and run a very long course before entering the peri-opticon. In the middle of the decussation there may be usually found one or more large nerve-cells, and a few others close up to the peri-opticon (fig. 23, *n. c.*). The peri-opticon is in Eristalis a continuous ganglion, and cannot be said to be composed of numerous separate elements.

In longitudinal sections (*v.* fig. 23) through the peri-opticon there is certainly an appearance somewhat similar to that of Musca (*v.* fig. 2, *p. op.*), but this is not due in Eristalis to its being composed of a number of cylindrical elements, but to the fact that it is perforated by bundles of thin-walled tracheal vessels (*v.* fig. 23, *t. v.*), which seem in such a section to divide it into elements. When a section is examined which is made in a plane at right angles to the direction of these vessels it is seen that the peri-opticon forms a continuous ganglion (Pl. XVII, fig. 24, *p. op.*), and that these vessels run through it in bundles (*t. v.*) situated at fairly even distances from one another. In fact we have in Eristalis a further stage in the complexity of the ganglion than we have in Aeschna, for the elements which in the latter have only partially fused together are in this form completely fused. In Eristalis, however, a system of tracheal vessels perforates this structure, a system which seems to be absent in Aeschna and the other forms we have so far considered.

In the silkworm moth (Bombyx), however, there is a solid homogeneous peri-opticon devoid of tracheal vessels.

Passing from the Hexapoda I will describe briefly the optic tract of Carcinus moenas as a type of Crustacean. A longitudinal section through the ophthalmic peduncle reveals the same three optic ganglia I have described in insects; the opticon (fig. 14, *op.*) is situated at the end of the optic nerve (*o. n.*), and this is connected by nerve-fibrils, which do not appear to decussate with the epi-opticon (fig. 14, *e. op.*), and this again by nerve-fibrils (*N. f.*), which completely decussate with the peri-opticon (*p. op.*).

The peri-opticon in Crustacea (Carcinus, Astacus, Homarus, Squilla) is a solid ganglion, similar to the epi-opticon, and cannot be separated into elements. The terminal anastomosis (fig. 14, *ta.*) is more complicated than it is in most insects (*ta.*), but the four regions I previously described may be recognised (1, 2, 3, 4).

I shall not continue here my description of the optic tract of Crustacea, as I hope soon to be able to collect my observations

upon this group into a separate paper; but I have said sufficient to show the relation that exists between the peri-opticon and terminal anastomosis of Crustacea and the homologous structures of the Hexapoda.

Before leaving this part of my subject I must refer to some observations I have made upon the development of these structures in the bee.

In a young bee, some time before it emerges from the cell, the fibrils passing from the epi-opticon to the peri-opticon do not cross one another (fig. 6, *Nf.*), but the decussation takes place at a later stage in a manner I have been unable to follow. I imagine, however, that is due to a shifting of the position of the fibrils at their origin from the epi-opticon, in a manner somewhat similar to that which occurs in Vertebrata when the roots of the posterior spinal nerve shift from the neural crest to a lateral position.

At first the fibrils pass straight from the epi-opticon to the eye, but an anastomosis soon takes place, and the limits of the peri-opticon are indicated by two rows of nerve-cells, by the presence of well-marked transverse and looped anastomosing fibrillæ, and by a thickening of many of the original fibrils (fig. 6). As development proceeds the thickened fibrils split up into masses of neurospongium, joined together by the transverse anastomosing fibrillæ, and each of these forms one of the elements of which the peri-opticon of the adult is composed. Before the bee is fully developed these elements stand some little distance apart from one another, as they do in Musca, but the spaces between them are gradually filled up by the development of new elements and the growth of those already formed.

To recapitulate, then, I have shown that in the young Periplaneta the optic nerve-fibrils which leave the peri-opticon pass without decussating to the ommateum; in the adult Periplaneta there is a partial decussation; and that in Nepa there is no decussation, but the anastomosis is complicated by the presence of looped and transverse anastomoses. In Musca the fibrils are split up into little cylindrical blocks of neurospongium, which

I have called the elements of the peri-opticon; in bees, wasps, and many Lepidoptera the elements of the peri-opticon are long, slender, and close-set; in Aeschna they have partially fused with one another; and in Bombyx, Eristalis, and the Crustacea they have completely fused to form a complete and continuous ganglion, similar in every way to the opticon and epi-opticon.

§ 3. Further Remarks upon the Histology of the Optic Tract.

In the optic tract of insects we can distinguish the following nervous structures: nerve-fibrils and fibrillæ, neurospongium, nerve-cells, and occasionally ganglion-cells. The nerve-fibrils are found in the regions between the opticon and epi-opticon, between the epi-opticon and the peri-opticon, and in the terminal anastomosis. They must be considered to be naked axis cylinders, are devoid of any medullary sheath, and frequently break up to anastomose freely with their neighbours. In the vicinity of the main ganglia they break up into a number of minute fibrillæ, which, anastomosing together, form a dense plexus or meshwork, which forms the main part of the ganglia, and which I have called a neurospongium. This connection between nerve-fibrils, fibrillæ, and neurospongium may be well seen in the epi-opticon of the young bee (Pl. XVII, fig. 25, *e. op.*). Each nerve-fibril seems to be composed of a number of very fine fibrillæ closely cemented together, and to be capable of splitting up into its component parts, where an intimate anastomosis is requisite or necessary. The different stages of the complexity of this anastomosis have been seen in the peri-opticon of the various insects I have described above. Thus, in Blatta the fibrils split up into finer fibrils in this region, which anastomose with one another, but the most ultimate division of each fibril seems never to occur. In Nepa the anastomosis is still more complicated, and in some cases the nerve-fibrils seem to split up into their ultimate fibrillæ. In Musca finally the subdivision of the fibrils is complete, and a true neurospongium is formed by the anastomosing fibrillæ.

In many cases it is difficult to make out, even after the most careful histological treatment, the reticulum of fibrillæ of the neurospongium of adult animals. In the adult A e s c h n a g r a n d i s, however, the nature of the neurospongium of the epi-opticon can be readily seen with high powers (fig. 22). The younger the animal, too, the coarser is the neurospongium ; thus in the very young Periplaneta or bee, the fibrillæ may be very readily seen (Pls. XV, XVII, figs. 6 and 25).

Nerve-cells are so very commonly met with in the central ganglia and elsewhere, and are very frequently crowded toge-ther, forming a kind of sheath embracing the other parts of the ganglia, that they must play a not unimportant part in the physiological processes of these parts. They were recognised as cells by Leydig (13), and described by him as such, but owing to the introduction of his term "Punkt-substanz" for the nerve-cell sheath, their true nature seems to have been over-looked or misunderstood by some of his successors.

It is true that in such forms as Musca, and many other adult insects, the cellular nature of the "Punktsubstanz" is not easy to make out, and it seems at first sight to be composed of numerous nuclei closely packed together. In the young bee, however, each nucleus can be seen to be encased in a delicate sheath of clear protoplasm which is drawn out into a number of fine processes which communicate with similar pro-cesses of other cells or with the nerve-fibrils. In the silkworm moth, the true cell-protoplasm is comparatively large (Pl. XV, fig. 15) in the adult, and as it stains well in borax-carmine and hæmatoxylon it can be readily seen.

Ganglion-cells differ from nerve-cells only in the relative amount of cell-protoplasm to nucleus. They are not usually found in the optic tract of insects, although they are present occasionally in some. The best ganglion-cells I have seen are in the brain of Periplaneta (Pl. XVII, fig. 23) and in such the cell-protoplasm is very considerable, and stains well. I have found in this region apolar, bipolar, and tripolar ganglion-cells (*a, b, c*.).

§ 4. The Comparative Anatomy of the Ommateum of the various orders of insects is, thanks to valuable monographs of Max Schultze (18), and Grenacher (7), so well known that it would be superfluous for me to go over the ground again. I have made careful series of sections through the eyes of many of the genera described by Grenacher, and I have been able to assure myself in almost every particular of the accuracy of his observations.

The question of the relation of the so-called pseudocone of certain insects to the well-known crystalline cone of others, is, however, one to which I have devoted some attention, and the result of my observations may not be devoid of interest.

The term "pseudocone" was given by Grenacher to the structure corresponding to the crystalline cones of the majority of insects and Crustacea, which is found in the following genera of the Diptera, Tabanus, Sarcophaga, Hæmatotopa, Syrphus, Musca, and I can add as a result of my own researches, Eristalis. The pseudocone, according to Grenacher, differs from the "eucone" and "acone" in possessing the following characteristics which I quote verbatim from his work (p. 88). "Während beim Acone Augen die vier hinter der Facette gelegenen und sie abscheidenden Zellen zeitlebens als solche unverändert persistiren; beim euconen aber ausser der Facette noch den aus ebensoviel Segmenten, als Zellen vorhanden sind, bestehenden Krystallkegel aussondern (und zwar erscheint jedes Segment ursprünglich im Innern der zugehörigen Zelle) : scheiden die vier Krystallzellen beim pseudoconen Auge eine weiche, halb oder ganz flüssige Substanz aus, die, zusammengehalten durch trichterförmig gestaltete Haupt (*tp.*) pigmentzellen, functionell dem Krystallkegel zuvergleichen ist.

"Sie ist aber vor den Zellen gelegen, durch deren Thätigkeit sie entstanden ist, zwischen denselben und der Facette ; die Kerne jener Zellen, die man als Semper'sche bezeichnet, liegen demnach nicht, wie bei den andern zussamengetzten Augen, der Facette stark genähert, sondern in einem oft recht erheblichen Abstand von ihr abgerückt."

According to this view then, the pseudocone is a space filled with a fluid or semi-fluid substance situated between the cornea and the " Semper's nuclei."

In Musca this space is seen to be traversed by four very delicate clear bands passing from the innermost regions of the pseudocone to the facet. (Grenacher, p. 90, figs. 63, 64, *Ps. C.*).

There can be no doubt now, I think, that these bands do not exist as such in the living eye, but that their appearance is due to the action of reagents. I have found them in a similar position in Eristalis, and I am led to believe that they represent the shrivelled remains of the external part of the cone-cells.

This portion of the cone-cell I believe to be filled in the living condition with a fluid or semifluid substance which performs the same function as the crystalline cone of the " eucone eyes," but that it is partially or wholly dissolved by reagents and only the cell walls, and perhaps part of the proto-plasmic cell substance are left in the form of four bands passing through the space formerly occupied by the pseudocone. The inner part of the cone-cells containing four nuclei (' Semper's nuclei' Grenacher) are seen at the base of the pseudocone in close contact with the end of the rhabdom. The development of the crystalline cone has been carefully investigated by Claparède (5), and he shows that it is developed in four pieces in the primitive cone-cells (Krystallzellen) on the inner side of the nuclei, that each part increases in size until it forms with the three others the crystalline cone by apposition. In the adult condition the crystalline cone is with difficulty divisible into its four constituent parts, and the nuclei of the cone-cells remain as the " Semper's nuclei " of the adult. From my own researches upon the developing bee and cock-roach. I believe this to be a very fair statement of what occurs. The structure of the adult crystalline cone may be very well studied in Aeschna grandis (figs. 12 and 13) in which the " Semper's nuclei " are seen to be large, well marked, and usually situated in the four corners of the quadrangular

protoplasmic mass, just behind the facet. It is not easy, either in the adult or developing condition, to see the outlines of the four cells composing this protoplasmic mass, but I have no doubt that if it were properly stained by nitrate of silver they could be easily demonstrated. Even in the adult the four portions of the cone are never completely fused, but delicate bands of protoplasm staining well in borax carmine remain between them throughout life.

If this description of the pseudocone and eucone eye is accurate, and I believe it so to be, the difference between the two, although fundamental, is not so excentric as it was formerly thought to be. The difference between them, I believe, lies in the fact that in the former the refracting body formed by the cone-cell lies behind the nuclei, and in the latter in front of it. In the acone eye, as Grenacher explains, the four primitive cone-cells remain, and no refracting body is developed in them at all.

The pigment of the ommateum of insects is usually very copious, and is supplied by cells whose protoplasm is drawn out into long processes, which sheath the ommatidia. Of these pigment-cells three series may be very generally recognised, although additional series are sometimes present, e. g. Lepidoptera. The three series are : 1. A series of pigment-cells, which ensheath the cone (fig. 3) and prevent extraneous rays of light from escaping; these may be called the "cone pigment-cells."

2. A series of pigment-cells situated in the outer region of the rhabdoms, which have long processes passing between the retinulæ and elsewhere, which may be called the external pigment-cells (fig. 2).

3. A series of pigment-cells usually resting upon the basilar membrane (fig. 3), whose processes pass externally between the retinulæ, and internally, in some cases, through the basilar membrane to the terminal anastomosis, and may be called the internal pigment-cells.

These three series of pigment-cells are very constant through the Hexapoda.

The basilar membrane is in all cases perforated by two sets of apertures, which are best seen in Agrion (Pl. XVII, fig. 28, *b.m.*); the larger apertures are for the passage of the tracheal vesicles, the smaller for the nerve-fibrils passing to the retinulæ.

The thickness of the basilar membrane varies considerably. In Agrion, Aeschna, and the Libellulidæ generally it is thick, but in the Diptera, Hymenoptera, and others very thin.

§ 5. The Distribution of Tracheæ to the Optic Tract and Ommateum.

The tracheæ of the insect may be divided under four heads : (1) large tracheal trunks lying in the various parts of the body ; (2) smaller tracheal vessels ramifying through the various viscera, with spiral markings on their walls ; (3) very thin-walled vessels devoid of spiral markings, ramifying in the various tissues and organs of the body, and in some cases anastomosing with one another ; and finally, (4) tracheal vesicles, first recognised by Strauss-Durckheim (20), as closed sacs or dilatations of the tracheal vessels. Speaking of them he says, p. 319, " Dans les vesicules trachéenes qui ne se rencontrent que chez un certain nombre d'insectes, le fil spiral n'existe point, et elles sont réduites a la tunique extérieure ; car l'intérieure ne s'y laisse aucunement apercevoir."

The extent to which spirally-marked tracheæ are present in the nerve-ganglia seems to vary enormously, as may readily be seen by examining sections through the brain and optic tract of various insects, or consulting the figures which have been published by Leydig (12) and others.

Taking the tracheæ of what I have called the terminal anastomosis alone, we find that in Eristalis the spirally-marked tracheæ in this region are very large and very numerous ; in fact they form a very dense network just behind the basilar membrane. In Musca they are also very numerous, but the network of them is not nearly so dense as it is in Eristalis. They are present in this region in Blatta and Dytiscus (Leydig), and in the Lepidoptera (Sphinx, Acherontia, Noctua), although

in this order the walls are very thin and the spiral markings indistinct. In Apis, Aeschna, Nepa, Agrion, &c., spirally-marked tracheæ are absent in this region. Again, in that region of the optic tract situated just behind the peri-opticon we find very few tracheæ in Musca, and in some individual examples I have found none at all, but in Eristalis they are very numerous (Pl. XVI, fig. 23), and even decussate with the optic nerve-fibrils. But although the spirally-marked tracheæ may be absent in many parts of the optic tract there is very good evidence for believing that in all cases it is copiously supplied with thin-walled tracheal vessels. Thus, in Aeschna and Agrion the region marked 2 in the terminal anastomosis is undoubtedly occupied by thin-walled tracheal vessels, and in the former genus the spaces (Pl. XV, fig. 7, *s.*) found in the peri-opticon are probably occupied by such vessels.

The difficulty of differentiating these vessels in hardened and stained sections is remarkable. Thus, the tracheal vessels found in the ommateum of Musca (fig. 2, *t. v.*; fig. 3, *t. v.*) are quite invisible in many of my sections, although when the fresh eye is examined they are most conspicuous. It is true that in one series I have, that was stained in a remarkably good hæmatoxylon fluid (*vide* infra), I was able to see them fairly well, and it was in this series that I was able to trace their connection with the tracheæ behind the basilar membrane (*vide* fig. 3, *T. tv.*); but, as a rule, they cannot be seen in hardened sections. I have turned my attention, however, more particularly to the distribution of the tracheæ in this region in Eristalis and Musca.

In both these forms two large tracheal trunks (Tt_1, Tt_2) are to be found at the sides of the optic tract lying in the groove between the epi-opticon and peri-opticon. The larger of these is external, the smaller internal. The larger one sends off special tracheæ to the terminal anastomosis, and does not send any branches into the peri-opticon or behind it. The smaller of them sends tracheæ into the optic tract behind the peri-opticon, never in front of it.

The tracheæ of the terminal anastomosis send off thin-walled

vessels devoid of spiral markings, which perforate the basilar membrane to end in the inter-ommatidial tracheal vesicles (fig. 3, *t. v.*). These vesicles are at the base broad, but taper to a point externally, and end in the region of the external pigment-cells.

In addition to these the tracheæ of the terminal anastomosis send off other thin-walled vessels, which perforate the peri-opticon and communicate with similar offshoots from the tracheæ situated behind it.

These cannot be seen very distinctly in Musca, but in Eristalis they are gathered together in bundles as they perforate the peri-opticon, and are easily distinguished by their comparatively thick walls (Pl. XVI, fig. 23).

§ 6. Historical and Critical.

The history of the research into the anatomy and development of the Arthropod eye practically dates from the publication of Johannes Müller's work 'Zur vergleichenden Physiologie des Gesichtssinnes' in 1826 (17). From that time to the publication of Grenacher's (7) great work 'Untersuchungen über das Sehorgan der Arthropoden' in 1879, the study was carried on by numerous investigators both in England and abroad, and much light was thrown upon the numerous branches of the subject.

It is not my purpose to enter here into the various points that have formed subjects for animated discussion in times gone by, nor is it my purpose to give any detailed account of researches previous to 1879, for all such information has been skilfully brought together and digested in the learned 'Historischkritische Uebersicht' of Dr. Grenacher's work. Since then, however, certain papers have been published describing certain structures in the eye and optic tract of insects, which cannot be allowed to pass by without some criticism.

The eye of the blow-fly was described by Grenacher (7, p. 90) in considerable detail, and the description I have given above differs from his only in a few minor points. He says, for instance, that the corneal facets are plano-convex, but they
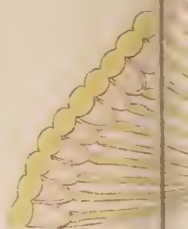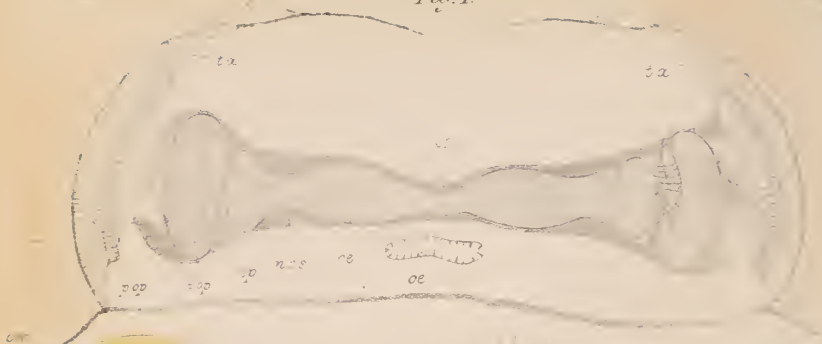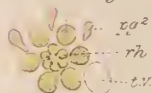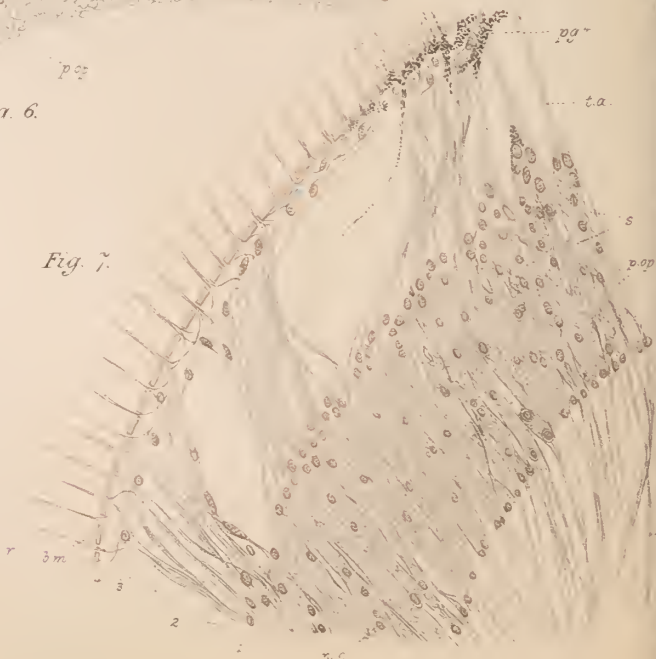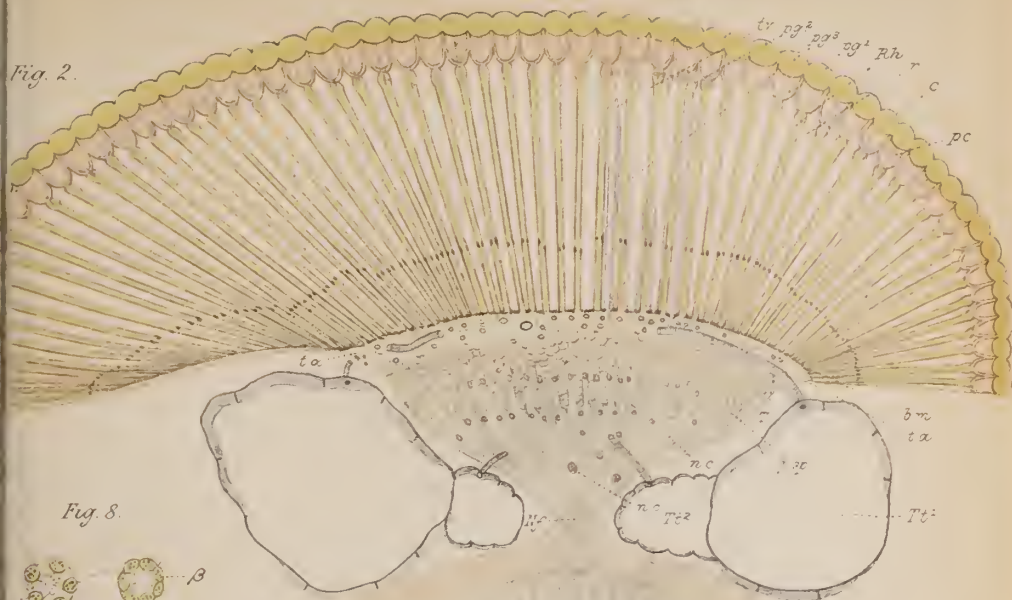
Fig. 1.

Fig. 3.

Fig. 4.

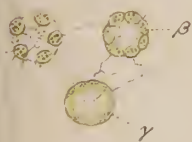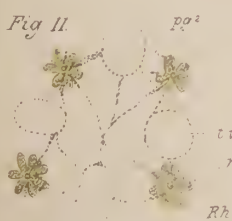Fig. 5.

Fig. 6.

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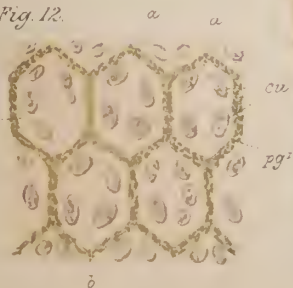S. J. Hickson del.

Fig. 2.

Fig. 8.
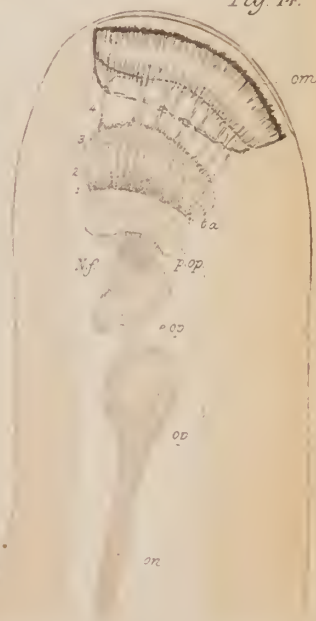
Fig. 9.

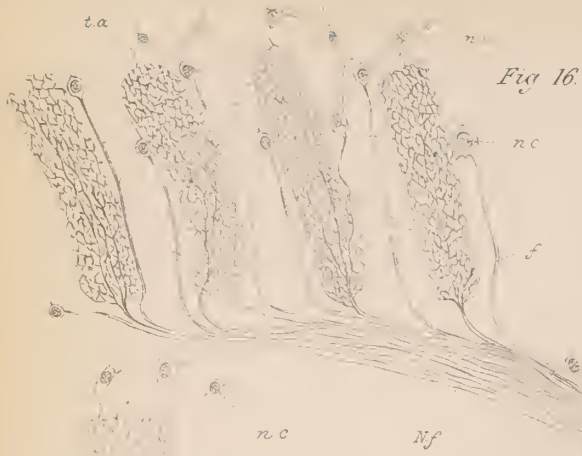Fig. 11.

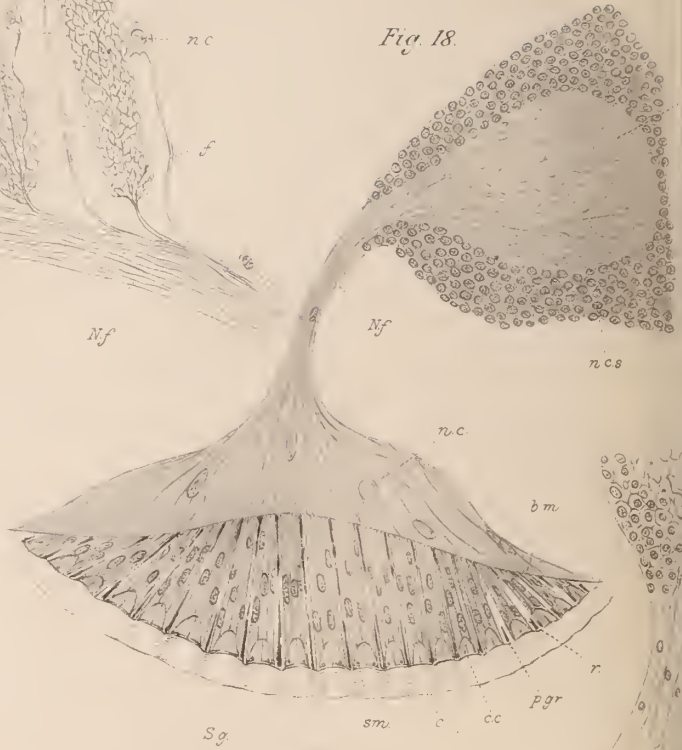Fig. 12.

Fig. 13.

Fig. 14.

Fig. 15.

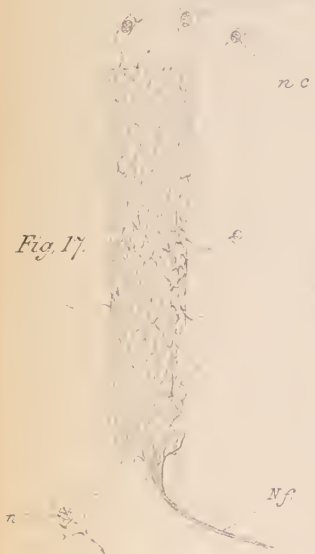Fig. 16.

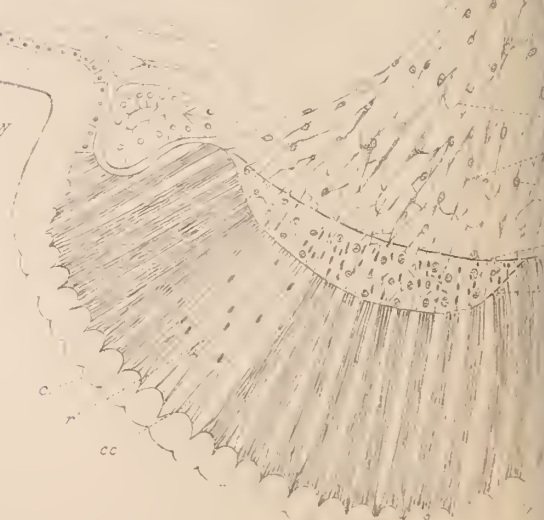Fig. 18.

Fig. 17.

Fig. 19.

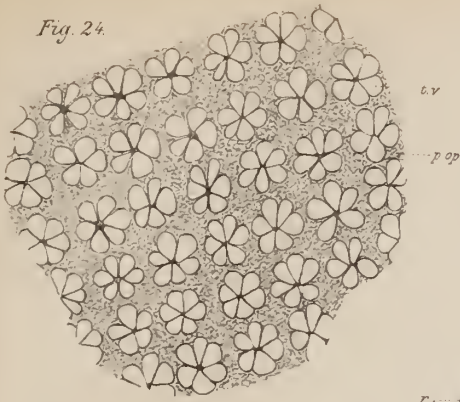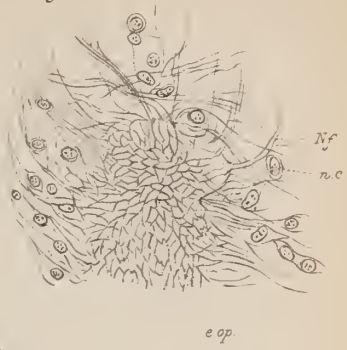Fig. 22.

Fig. 20.

Fig. 21.

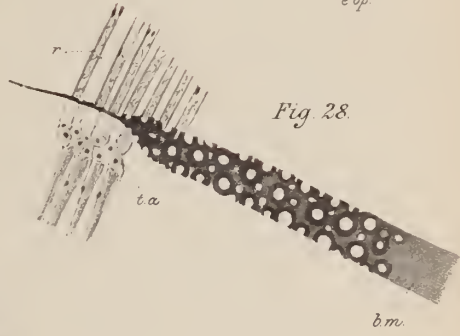Fig. 23.

Fig. 24.

Fig. 25.

Fig. 26.

Fig. 28.

Fig. 27.

Fig. 29.

Fig. 30.

Fig. 31.

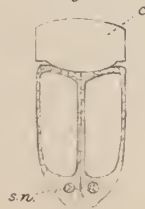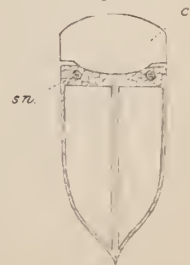Fig. 32.

Fig. 33.

Fig. 34.

Fig. 35.

are in reality biconvex, the plano-convex appearance being seen only when the sections are cut not quite perpendicularly, or do not pass quite through the centre of the facets. Carrière (3) figures the facets of Musca plano-convex. Lowne (15), whilst figuring them biconvex as I have done, places them amongst what he calls the "kistoid corneæ." The "'kistoid cornea' consists of a chitinous (?) articular membrane folded on itself so as to resemble a piece of honeycomb, the openings of the hexagonal cells being turned inwards." "The cavity of the corneal cell is occupied by the oil-like lens." I have never yet been able to find anything that would answer to this description. I have never found anything like "an oil-like lens," nor can the cornea be truly compared with a honeycomb. Each facet in Musca is formed of chitin, the outer layers of which are the hardest and do not stain, whilst the inner ones are softer and frequently take up a considerable amount of hæmatoxylon or borax carmine staining. I have not made a special study of the development of the cornea, but since the publication of Mr. Lowne's paper, I have re-examined many of my preparations of adult and immature eyes. As a result of this, I must express a very strong opinion that the facets are not developed from "four original cells," but from a continuous sheath of protoplasmic substance which underlies the whole surface of the cornea, and which remains as a living protoplasmic lamina until the eye is fully developed and then shrivels up. That the so-called "Semper's nuclei" have nothing whatever to do with the formation of the facets, I should think has been amply proved by the researches of Claparède (5), Max Schultz (18), and Grenacher (7).

My description of the ommatidia given above is almost identical with that of Grenacher's; the only point in which I am inclined to differ from him is in his description of the nuclei of the retinulæ.

He says : " Von den Kernen der Retinula liegen sechs im vordern Drittel, wo sie (an gehärteten Augen) buckelartige Auftreibungen verursachen, der siebente findet sich immer im hintern Drittel und gehört wohl zum Centralstäbschen."

Carrière (3) says that only five nuclei lie in the uppermost end of the retinulæ. My own researches lead me to believe that six nuclei are found in the uppermost part which belong to the six retinulæ, and that the one or two nuclei which are found about half way down are additional nuclei belonging to one or two of these six retinulæ. In other words, I believe that some of the retinulæ possess two nuclei, and my opinion upon this point is supported by the investigation of the same structures in other Insects and Crustacea, and by the researches of Claparède and Weissman upon their development. I cannot see anything morphologically monstrous in supposing that the retinula cells possess more than one nucleus.

The inter-ommatidial tracheal vesicles have been known to exist for some time. They were mentioned by Swammerdam, who said : " Es ist wunderbar, wie und in was für einer grossen Menge die Luftröhren . . . . hinaufklimmen," by Serres, by Will, and by Leydig (11, 12). Grenacher (7) figures a portion of one of them in Tabanus bovinus (Taf. vii, fig. 60), and they are mentioned by Lowne. By some authorities they have been supposed to perform the function of a tapetum owing to their brilliant glittering appearance in the living eye, but the sheath of pigment-cells surrounding the ommatidia, and between them and the tracheal vesicles, militates against this view; and they must be regarded, I think, simply as vessels for the aeration of the ommateal tissues. They were also known to Ciaccio (4), whose description of the distribution of the tracheæ to the optic tract in the Diptera is exceedingly accurate. Berger (2) also mentions the tracheal diverticula, but does not figure them.

The function of the retinulæ has been a subject which has been very largely discussed by the older writers, but the balance of opinion has always been in favour of considering the retinulæ to be the true nerve-end cells. This was most certainly the opinion of Müller (17), but although this was combatted by Gottsche (6), and by Wagner (21), who thought he could trace the nerve-fibrils through the retinulæ to the

crystalline cones, in 1864 Leydig was able to express a very strong opinion that the retinulæ (Nervenfäsern) are the nerve-end cells and " dass man die Analoga nicht in den gewöhn-lichen Nervenprimitivfasern der Wirbelthiere suchen darf, sondern einzig und allein in den Bacillis und Conis der Retina höherer Thiere."

The same opinion was expressed in 1868 by Max Schultz (18), who at the close of his exceedingly careful and valuable work on the compound eyes of Crustacea and Insects, says that it is a proved fact that the retinulæ (Sehstäben) are the end-organs of the optic nerves.

In 1879 Dr. Grenacher, after a prolonged and laborious re-investigation of the whole subject, came to the same con-clusion, and for the first time introduced the term " retinulæ " for the structures which are now almost universally known by that name.

After the subject had thus been thoroughly investigated and re-investigated by such celebrated naturalists, and the results of such investigations had led to conclusions which in all important respects were identical, it might have been thought that the matter was definitely settled.   However, a short paper was published last year in the ' Proc. Roy. Soc.,' by Mr. Lowne (15), in which a view was put forward that the retinulæ are not the nerve-end cells at all, but that the true retina is situated behind the basilar membrane.   Lowne's retina is situated in Musca in a position identical with my peri-opticon, and I have no doubt that he has mistaken the elements of this ganglion for cells, just as Carrière (3) did, and supposed that they corresponded with the retinal cells of other animals.   But this view is perfectly untenable, for not only does anatomy teach us that the optic nerve-fibrils end in the retinulæ, but morpho-logy teaches us that they are homologous with the nerve-end cells of the eyes of other animals, and the few physiological experiments that have been made also show that they are eminently adapted for light-perceiving purposes.

If anything further were required to prove that the retinulæ are homologous with visual nerve-end cells of other animals, it

is the fact that Leydig (11) discovered that they are possessed of a true retina purple. As this substance is not known to occur anywhere in the animal body but in the visual nerve-end cells, and as the retinulæ of Arthropods possess this substance and Lowne's bacillar layer does not, the retinulæ must be the nerve-end cells of the Arthropod eye.

This being the case, the terms "Dioptron," and "Neuron," "Retina," and "Great rods," introduced by Lowne, must be rejected, and the nomenclature used which has been introduced by Grenacher (7), and Lankester and Bourne (15).

The first important paper upon the optic tract of Arthropoda was by Emile Berger (2), who described and figured this region in the larva of Libellula, in Musca, Dytiscus Apis, Pieris, Squilla, Astacus, &c.

He was the first to describe the true position of the optic nerve, namely, between the opticon and the cerebrum ; and he also figured, fairly accurately, the epi-opticon and the optic nerve-fibrils decussating between this and the peri-opticon. The rest of the optic tract he divided as follows. The optic nerve-fibrils, after they have decussated, enter a ganglion-cell layer, this is followed by a molecular layer (Marksubstanz), which is situated immediately behind a nuclear layer (Körner-schicht), which is separated from the basement membrane of the ommateum by a "nerve-bundle sheath." These various layers, however, are not so constant as he supposed, nor are they in all cases just as he described. The ganglion-cell layer is not always present, and sometimes it is only represented by a few scattered cells as in Eristalis, nor can any distinction be drawn between the cells situated in this region and the region situated in front of the peri-opticon. In both cases the cells are nerve-cells and not ganglion-cells, according to the distinction I have drawn between the two ; that is, in neither region are cells found containing a considerable quantity of true cell-protoplasm.

The molecular layer of Berger corresponds with my peri-opticon. He does not seem to have noticed that it is composed of a number of elements, but his idea that it corresponded in

structure with the substance of the epi-opticon and opticon shows that he was able to appreciate its histological nature as far as possible, and he did not fall into the error of supposing, as some subsequent observers have done, that it contained bacillar or cubical cell-elements. The nuclear layer and the "nerve-bundle" sheath of Berger together make up what I have termed above the "terminal anastomosis."

Previous to the publication of Berger's paper, the optic tract of Insects had been briefly described and names given to the various regions. Thus Weissmann (21) called the opticon and epi-opticon the "bulbus," the region where the optic fibrils decussate the "Stiel," and the peri-opticon the "Augenscheibe." Leydig (2) described the three ganglia in Formica as the "kleiner Kern des Sehlappens," "grosser Kern d. S.," and "dritter Kern, d. S," and in Dytiscus as the "erster," "zweiter," und "dritter" nuclei. Ciaccio (4) describes the following layers of the retina : 1. A "membrana limitans" posterior. 2. The layer of optic nerve-fibrils. 3. The layer of nerve-cells. 4. The "membrana limitans" anterior. 5. The layer of rods. In fact, Ciaccio anticipated Berger's idea, that the retina of insects contains a number of nerve-layers as the Vertebrate retina does, but was unable to give it the minute histological analysis it deserved.

Since Berger's paper appeared Carrière (3) has described the peri-opticon as a layer of "long, palisade-shaped cells, the number of which corresponds with that of the eye units. Every one of these palisade-cells possesses an oblong nucleus at its foremost somewhat broader end." My researches show that this description is quite inaccurate. The elements of the peri-opticon are not cells, and the large oval nucleus situated in each element which Carrière figures does not exist. Nerve-cells, when they exist in the region of the peri-opticon in Musca, lie between the elements, and not in them, as my figures show.

In a young Sarcophaga carnaria Carrière found "a refracting chitinous or cuticular tube, which lies in the midst of the 'palisade-cell,'" and he finds in Musca vomitoria

that each palisade-cell has a cylindrical axis, which he says may be such a chitinous tube.

I have examined my sections of Musca, both transverse and longitudinal, very carefully, but can find no evidence of the existence of any such tube, and I have also, by a process of maceration described below, satisfied myself that no chitinous structures of this kind are present in the optic tract behind.

The tubes of Sarcophaga may possibly be tracheal tubes similar to those I have described in the peri-opticon of Eristalis, and the appearance which Carrière describes (but does not figure) in Musca may be due to the curious effects which the pigment often produces (v. figs. 8 and 9).

The recent paper by Lowne upon the retina of insects I have referred to above and criticised elsewhere (8). My description of the optic tract of insects is quite different from his, as is my opinion of the function of the various parts.

§ 7. Some general Considerations upon the Eye and Optic Tract of Insects.

The layer composed of the retinulæ and rhabdoms cannot, as Ciaccio (4), Berger (2), and others have shown, be considered to be the equivalent of the retina of other animals. It is only part of the retina, namely, that part of it which bears the nerve-end cells, and corresponds, functionally at any rate, to the layer of rods and cones of the Vertebrate eye. In Vertebrates and in those Invertebrates with large, highly-organised eyes, such as Cephalopods and such genera as Pecten and Spondylus among Lamellibranchs, and Alciope among the Polychæta, the retina contains, in addition to the rods and cones, several layers of nerve-cells, ganglion-cells, neurospongium, &c., which are very different in order and arrangement for different groups of animals, but very constant in the individual genera and species.

In Arthropoda we also find behind the basilar membrane of the ommateum similar complicated nervous strata, which in all probability perform the same function.

We cannot, however, trace the same layers of nerve-cells, ganglion-cells, &c., layer for layer, in Arthropoda, that we find in the retina of Vertebrata, any more than we can trace them in Cephalopods, in Alciope, or in Pecten. All that we can say is that there is present in all animals with highly-organised eyes certain complicated nervous structures situated between the nerve-end cells and the brain which are probably for the purpose of elaborating and combining the sensations received by the nerve-end cells.

How much, then, of the optic tract of insects should be considered to belong to the retina? It seems to me that to be consistent we should consider all the nerve structure lying between the crystalline cone-layer and the true optic nerve to be analogous with the retina of other animals.

According to my view, in fact, the retina of insects consists of the retinulæ, peri-opticon, epi-opticon, opticon, and all the intermediate nerve-tracts.

Both Ciaccio (4) and Berger (2) considered the retina proper to end at the region of the decussating fibres (N. f.). If this is the case the decussating nerve-fibrils should represent the optic nerve, which they do not, as Berger himself has shown.

The true optic nerve lies between the opticon and the cerebral ganglion, and it is this which is so enormously elongated in the podophthalmatous Arthropoda.

It may seem strange at first to have to consider those structures in the eyes of Arthropods, which have hitherto been somewhat loosely described as " optic ganglia," to be really part of the gigantic retina; but if the nervous layers of the retina are really for the purpose of combining and elaborating the impressions received by the nerve-end cells, as I suppose they must be, it is only natural to find them larger and more complicated in the Arthropod eye than in the Vertebrate eye.

In the Vertebrate eye the nerve-end cells are, comparatively speaking, extremely small and very closely packed, and consequently the nervous effort of translating the sensations of the nerve-end cells into a picture is very much less than it is in

the Arthropods, where the nerve-end cells are comparatively very large and widely separated from one another.

In the human eye, for example, the distance between the centres of two adjacent cones is only $\frac{4}{1000}$ mm., but in Musca the distance between adjacent ommatidia is $\frac{1}{100}$ mm. In fact the picture, as received by the nerve-end cells of the Vertebrate eye, is much more complete in itself than it can possibly be in any Arthropod eye, and consequently the latter possesses a much more elaborate and complete translating apparatus in their retina than the former possesses.

If this view be a sound one it is necessary to give up the term " optic ganglion," as applied to terminal ganglionic swellings in the optic tract of animals, such as the so-called optic ganglia of Cephalopods and of Arthropods, and consider them all as forming part of the true retina.

The observations I have made concerning the comparative anatomy and development of the peri-opticon may possibly possess an interest outside the bounds of comparative ophthalmology.

In considering the origin of the nervous system of animals we are able to see fairly clearly the origin of nerve-fibres and ganglion-cells. Thus Balfour (1) said : " From embryology we learn that the ganglion-cells of the central part of the nervous system are originally derived from the simple undifferentiated epithelial cells of the surface of the body ;" " that ganglion-cells have been evolved from simple epithelial cells of the epidermis ;" and that " the primitive nerves were outgrowths of the original ganglion-cells ;" but the evolution of the central ganglia has not hitherto been investigated.

It is, I think, evident, from both embryological and phylogenetic considerations, that the formation of the central ganglia was subsequent to that of the ganglion-cells and nerve-fibres, and that they must have originated from either one or both of these elements.

The characteristic feature of a ganglion is the very fine reticulum of nerve fibrillæ which forms what I have called a neurospongium, with which are associated a number of larger

nerve-fibrils, and a number of nerve- and ganglion-cells. The evidence I have brought forward by my researches upon the phylogeny and development of the peri-opticon of insects leads me to suppose that the neurospongium is formed by a breaking up of the nerve-fibrils into a number of ultimate fibrillæ which, anastomosing with one another, form the neurospongium. I have not been able to turn my attention to the development of the central ganglia in other animals, so I am unable to say definitely that this is the case universally throughout the animal kingdom, but it is very probable that it is so, and that we have in the various forms of peri-opticon an indication of the manner in which central ganglia were primarily evolved from the primitive nerve-fibres and ganglion-cells which preceded them.

§ 8. Methods.

As a short description of some of the methods I have employed in studying the eyes and optic tract of Arthropods may be useful to those engaged in similar researches, I propose in this section to mention a few of those that I have found most useful and have given the best results. For making sections through the eye of M u s c a v o m i t o r i a I have found it best to dissect away the posterior wall of the cranium of the fresh insect and then to expose it to the fumes of 1 per cent. osmic acid solution for forty minutes, then to wash in 60 per cent. spirit for a few minutes, and finally, to harden in absolute alcohol. Crania thus prepared may be cut into fine sections by the automatic microtome, and stained in hæmatoxylin or borax carmine. With most insects, however, I have found it impossible to use this microtome owing to the hardness of the chitin of the cranium and of the mouth appendages. In such cases I have used a Jung's microtome with the razor set so as to give a long sweep at each stroke, and the sections carefully removed from the razor, and mounted one by one.

I have tried various methods for depigmenting the eyes such as bleaching powder, nitric acid, chlorine, &c., but the best is that of exposing the sections when cut to the action of nitrous

fumes. This is done in the following manner. The sections are fixed in position on the slide by Meyer's albumin and glycerine solution, and when the paraffin has been removed by turpentine and the turpentine driven off by absolute alcohol, the slide is inverted over a capsule containing 90 per cent. spirit, to which a few drops of strong nitric acid have been added. Copious nitrous fumes are given off and the pigment dissolves. The action can be stopped at any moment by washing with neutral spirit and when the washing is complete the sections can be stained in hæmatoxylon or any other solution.

For teasing the best solution is chloral hydrate. I leave the eye or optic tract in a 5 per cent. solution of chloral hydrate for twenty-four hours, and then tease with needles and mount in glycerine. In some cases I have made very satisfactory preparations by fixing the teased tissues to the slide with albumin and glycerine solution and then washing with spirit and staining in the ordinary way, or staining after depigmenting with nitrous fumes.

I have tried various kinds of hæmatoxylon stains, but the solution which gives the best results, and is in every way the most satisfactory, is one which I have made by following Mitchell's (16) instructions, with a few additional precautions. I will describe here the mode in which I now make hæmatoxylon stain.

Take 56 grammes of the logwood extract and thoroughly pound it in a mortar. Then place it on a filter, and pour about a litre and a half of ordinary tap water through it. The filtrate may be thrown away and the residue allowed to dry. In the meantime prepare a solution of alum as follows :—Take 25 grammes of alum, and after they have been thoroughly pounded in a mortar pour them into 250 cc. of distilled water. To this solution add strong potash until a precipitate is formed, which will not dissolve upon stirring and standing.

Pour the alum solution thus made on to the hæmatoxylon residue, and allow them to macerate together for three or four days in a warm room. Then filter the hæmatoxylon solution into a bottle provided with a closely-fitting stopper, and add to it 10 cc. of pure glycerine and 100 cc. of 90 per cent. spirit.

(The residue need not be thrown away, for it can be macerated again with alum solution for a week or more, and a good strong stain obtained as before.) When the solution is thus made it should be well shaken, and allowed to stand for some weeks before being used. This solution of hæmatoxylon improves considerably with age. The oldest I have was made about twelve months ago, and is by far the best.

The hæmatoxylon stain produced by this recipe possesses several advantages over others. In most cases it differentiates the tissues admirably; nuclei stain deeply, cell protoplasm faintly; it seems to last a long time without showing signs of fading, and, as it penetrates well, it is very useful for staining in bulk.

§ 9. Summary.

In this memoir, then, I have described in detail the eye and optic tract of Musca vomitoria. The pseudo-cones I have found to be composed of four cells with their nuclei situated internally, each one containing a large watery or albuminous vacuole, which serves the same purpose, and is morphologically homologous with the crystalline cone of the "eucone" eyes. There are six retinulæ cells, each possessing a nucleus situated in that part of it which lies immediately behind the pseudo-cones, and in some cases an additional nucleus, situated about half way down. I have figured for the first time the inter-ommatidial tracheal vesicles which have been previously observed by several investigators. In the optic tract I have described three ganglia—the opticon, epi-opticon, and peri-opticon. The last of these is composed of a number of small cylindrical elements of a tissue composed of a sponge work of nerve-fibrillæ, which I have called a "neurospongium." The opticon and the epi-opticon are present in all insects and in most of the higher Crustacea. The peri-opticon appears comparatively late in development, but is never found even in the adults of Periplaneta and Nepa.

The peri-opticon, when present, is usually composed of a number of cylindrical elements, which partially fuse in Aeschna

and completely in Eristalis, Bombyx, and the Crustacea. In Eristalis the peri-opticon is traversed by a number of delicate tracheal vessels.

The terminal optic anastomosis of Nepa is more complicated than it is in Periplaneta, and seems to be an intermediate stage between the simple anastomosis and the true peri-opticon of Musca.

A similar series of intermediate stages between the simple anastomosis and a true peri-opticon has been traced in the development of these parts in the Bee.

The development and comparative anatomy of the peri-opticon of insects is interesting, as it may indicate the mode in which central ganglia were first formed from primitive nerve-fibrils and cells.

My researches seem to me to corroborate the opinion of the majority of previous investigators, that the retinulæ are the true nerve-end cells.

My researches were carried on entirely in the morphological laboratory at Oxford, and I have to thank Professor Moseley for much valuable help and advice, and to Professor Lankester for many valuable suggestions.

§ 10. Bibliography.

The following list does not pretend to be a complete bibliography of the subject, but it contains the most important of the works I have consulted in preparing this memoir. For further information I must refer to Grenacher (7).

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DESCRIPTION OF PLATES XV, XVI, & XVII,

Illustrating Dr. Hickson's Memoir on "The Eye and Optic Tract of Insects."

Reference Letters throughout.

b. m. Basilar membrane.   *c.* Cornea.   *ce.* Cerebrum.   *co.* Commissure. *c. c.* Crystalline cone.   *e. op.* Epiopticon.   *n.* Nucleus.   *Nf.* Optic nerve-fibrils between the epiopticon and periopticon.   *n. c.* Nerve-cell.   *n. c. s.* Nerve-cell sheath.   *œ.* Œsophagus.   *om.* Ommateum.   *o. n.* Optic nerve. *op.* Opticon.   *pc.* Pseudocone.   *p. op.* Periopticon.   *pg.$_1$, pg.$_2$, pg.$_3$.* Pigment-cells.   *r.* Retinulæ.   *Rh.* Rhabdom.   *rh.* Rhabdomere.   *Tt.$_1$.* Large tracheal trunk in the head.   *Tt.$_2$.* Smaller tracheal trunk.   *T.* Tracheæ with spirally-marked walls.   *t. v.* Thin-walled tracheal vessels and vesicles.

Fig. 1.—Diagrammatic vertical section through the head of Musca vomitoria, showing the relative positions of the cerebral and optic ganglia. *ce.* Cerebral ganglion.   *co.* Commissure between the cerebral ganglia.   *o. n.* Optic nerve.   *op.* Opticon.   *e. op.* Epiopticon.   *p. op.* Periopticon.   *t. a.* Terminal anastomosis.   *om.* Ommateum.   *n. c. s.* Nerve-cell sheath.   *œ.* Œsophagus.

Fig. 2.—Vertical section through the eye and optic tract of Musca vomitoria. In the greater part of the ommateum the pigment-cells have been omitted to prevent confusion, but the pigment-cells are drawn in the centre of the figure at *pg.$_1$, pg.$_2$, pg.$_3$.* In a similar manner the tracheal vesicles between the ommatidia are only inserted in two instances, *t. v. Tt.$_1$.* The large tracheal trunk which sends the branches to the terminal anastomosis, *t. a.*, to supply the tracheal vesicles of the ommateum.   *Tt.$_2$.* The smaller tracheal trunk which supplies branches to the optic tract behind the periopticon.

Fig. 3.—Ommatidium of Musca, semi-diagrammatic.   *c.* Cornea.   *pc.* Pseudocone.   *pg.$_1$.* Pigmented cells surrounding the pseudocone.   *pg.$_2$.* Additional pigment-cells.   *pg.$_3$.* Basal pigment-cells.   *n. pc.* Nuclei of pseudocone.   *r.* Retinulæ.   *n. r.* Nucleus of retinulæ.   *Rh.* Rhabdom.   *b. m.* Basilar membrane.   *T.* Trachea.   *tv.* Tracheal vesicle.   *t. a.* Terminal anastomosis sending fibres to the retinulæ.

Fig. 4.—Transverse section through the retinulæ and rhabdom in their outer region, showing the six retinulæ (*r.*) free from one another, and the rhabdom composed of six rhabdomeres fused together (*rh.*). The processes of the pigment-cells and their relation to the retinulæ are shown at *pg.$_2$*, and the relative size and position of the tracheal vesicle in this region is shown at *t. v.*

Fig. 5.—Transverse section of the same in the inner region, *i. e.* near the basilar membrane. The six retinulæ become fused into a tube ensheathing the rhabdom. The tracheal vesicle, *t. v.*, is very much larger proportionately.

Fig. 6.—Section through the periopticon of a young bee. *e. op.* epiopticon. *Nf.* Nerve-fibrils not decussating in this region at this stage. *p. op.* Periopticon indicated by a row of nerve-cells in front and behind, and by a thickening of the longitudinal fibrils, and by the characteristic looping transverse anastomoses. *t. a.* Terminal anastomosis. *n. c. s.* Nerve-cell sheath.

Fig. 7.—A small portion of the periopticon and terminal anastomosis of Aeschna grandis. *Nf.* The optic-nerve fibrils beyond their decussation. *p. op.* The periopticon, the elements of which are incompletely fused. *t. a.* The terminal anastomosis, showing four not very well-marked regions, 1, 2, 3, 4. *pg.4.* Pigment-cells and their branches, situated behind the basilar membrane. *b. m.* Basilar membrane. *r.* The retinulæ. The pigment-cells and their branches are omitted in the lower part of the figure. *s.* Spaces in the periopticon.

Fig. 8.—Transverse section through three elements of the periopticon of a blow-fly, showing the pigmentation of the outer region which is occasionally present. At *α*, the pigmentation has taken the form of five pillars; at *β* and *γ*, the form of a hollow cylinder. All of these varieties were found in one specimen.

Fig. 9.—Longitudinal section of the same, showing that this irregular pigmentation sometimes gives the appearance of small rods in the periopticon.

Fig. 10.—One of the outer pigment-cells. *n.* Nucleus. *in.* Internal process. *ex.* External process.

Fig. 11.—Transverse section through a portion of the ommateum of Aeschna grandis in the region of the retinulæ and rhabdoms. *r.* Retinulæ. *Rh.* Rhabdoms. *pg.2.* Branches of the outer pigment-cells. *t. v.* Tracheal vessels of the ommateum.

Fig. 12.—Transverse sections through a portion of the ommateum of Aeschna grandis, showing the relations of Semper's nuclei, *s. n.* They are usually four in number (*a. a.*), but occasionally five are to be seen (*b.*), and usually regular in position (*a. a.*), but sometimes irregular (*c.*).

Fig. 13.—Two crystalline cones (*c. c.*) of Aeschna grandis, showing the position of Semper's nuclei (*S. n.*) and the investing pigment-cells, *pg.1.*

Fig. 14.—Longitudinal section through the ophthalmic peduncle of Carcinus moenas. *o. n.* Optic nerve. *op.* Opticon. *e. op.* Epiopticon. *Nf.* Decussating fibrils between the epiopticon and periopticon. *p. op.* Periopticon, a solid ganglion like the epiopticon. *t. a.* Terminal anastomosis very extensive and complicated, but capable of being separated into four regions, 1, 2, 3, 4. *om.* Ommateum.

Fig. 15.—A small piece of the nerve-cell sheath of the brain of Bombyx mori (imago), showing the nuclei and cell protoplasm, in this form very con-

siderable, of the cells. Between the cells may be seen the nerve-fibrils anastomosing with one another and with the cell processes.

Fig. 16.—Four elements of the periopticon of Musca vomitoria, showing their connection with the optic fibrils (*Nf.*) internally, and with the nerve plexus and nerve-cells (*n. c.*) of the terminal anastomosis externally. Some of the optic fibrils (*f.*) run right through without breaking up into a neurospongium.

Fig. 17.—One of the elements of the periopticon of Musca vomitoria more highly magnified, showing the course of the nerve-fibrils through the neurospongium, and the relation of the fibrils of the neurospongium to the nerve-cells.

Fig. 18.—Section through the eye of a very young Periplaneta. *e. op.* Epiopticon. *n c. s.* Nerve-cell sheath. *Nf.* Optic nerve-fibrils, leaving the epiopticon without decussating. *n. c.* Nerve-cells in the terminal loose anastomosis. *b. m.* Basilar membrane. *r.* Retinulæ. *c. c.* Crystalline cone. *c.* Cornea incompletely faceted in the middle, unfaceted at the periphery. *pg.2.* Pigment-cells between the ommatidia.

Fig. 19.—Section through the eye of a full-grown male Periplaneta. *op.* Opticon. *e. op.* Epiopticon. *Nf.* Optic nerve-fibrils, partially decussating in the adult. *n. c.* Nerve-cells in the terminal anastomosis, which is here much denser and more complicated than it is in the young cockroach. *b. m.* Basilar membrane. *r.* Retinulæ.

Fig. 20.—Section of the eye and optic tract of Agrion bifurcatum, showing the general arrangement of the parts. *o. n.* Optic nerve. *op.* Opticon. *e. op.* Epiopticon. *n. c. s.* Nerve-cell sheath. *Nf.* Optic nerve-fibrils t the point of their decussation. *p. op.* Periopticon. *t. a.* Terminal anastomosis. *b. m.* Basilar membranes. *om.* Ommateum.

Fig. 21.—Periopticon and terminal anastomosis of Agrion bifurcatum more highly magnified than Fig. 20, showing the character of the elements of the periopticon, *p. op.*, and the structure of the terminal anastomosis. *t. a.* 1. The first layer of the terminal anastomosis, consisting of a plexus of fibrils and nerve-cells, *n. c.* 2. The second layer, in which the fibrils are collected together in bundles. 3. The final optic plexus and nerve-cells. 4. The layer in which the optic fibrils are collected in bundles to be distributed to the retinulæ (*r.*).

Fig. 22.—The eye of an adult Nepa cineraria. *e. op.* The epiopticon. *Nf.* The optic nerve-fibrils, going to form the terminal anastomosis. *p. m.* Perforated membrane through which this anastomosis passes. *ll.* Loopings or recurrent anastomoses of the nerve-fibrils. *om.* Ommatidia covered with the branches of the pigment-cells. In the centre of the ommateum three ommatidia are figured without their accompanying pigment, in order to show the retinulæ (*r.*) and the crystalline cone (*c. c.*).

Fig. 23.—Section through the epiopticon, periopticon, and terminal anastomosis of Eristalis lupinus. *e. op.* Epiopticon. *Nf.* Nerve-fibrils decussating between the epiopticon and the periopticon. *p. op.* The periopticon

here formed of a continuous neurospongium, perforated by numerous thin-walled tracheal vessels. The tracheal vessels passing through the periopticon are only represented on the right-hand side of the figure, they pass from the thick-walled spirally-marked branching tracheæ (T) behind the periopticon to similar tracheæ situated in front of it. The tracheæ passing in front of the periopticon are supplied by the large tracheal trunk, $Tt._1$, whereas those passing behind are supplied by the smaller tracheal trunk, $Tt._2$ Rh. The rhabdom. r. The retinulæ. $b.\ m$. The basilar membrane. $pg._3$. The internal pigment-cells.

Fig. 24.—Transverse section through the periopticon of Eristalis lupinus, × 600 diam. $t.\ v$. The bundles of tracheal vessels perforating the neurospongium of the periopticon, $p.\ op$.

Fig. 25.—A portion of the epiopticon of a young bee, with nerve-fibrils proceeding from it and the nerve-cells connected with them. The neurospongium of the epiopticon ($e.\ op$.) is composed of a large number of very fine fibrillæ, forming a dense meshwork and combining together in bundles to form the nerve-fibrils ($Nf.$) which pass out of it. The nerve-cells ($n.\ c$.) consist of a large nucleus surrounded by a delicate investment of cell protoplasm, which gives off fine branches communicating with the neurospongium and the fibrils.

Fig. 26.—A small portion of the epiopticon of an adult Aeschna grandis, showing the character of the neurospongium and the relative size and position of the nerve-cells ($n.\ c$.) and nerve-fibrils ($Nf.$).

Fig. 27.—Ganglion-cells from the brain of an adult male Periplaneta orientalis. a. A tripolar ganglion-cell. b. A bipolar cell. c. An apolar cell.

Fig. 28.—Portion of the basilar membrane of the eye of Agrion bifurcatum, showing the perforations, the larger ones for the tracheal vessels and the smaller ones for the terminal optic fibrils. r. Retinulæ. $t.\ a$. Terminal anastomosis.

Figs. 29—32.—Diagrammatic figures of the four principal varieties of periopticon met with in the Hexapoda. The nerve-cells are omitted.

Fig. 29.—The condition in Blatta in which the fibrils simply split up and anastomose.

Fig. 30.—The condition in Nepa and the developing bee, in which the anastomosis is complicated by fine looped and transverse anastomoses.

Fig. 31.—The condition in Musca in which the fibrils split up into their ultimate fibrillæ and these anastomose to form a true neurospongium.

Fig. 32.—The condition in Crustaceæ in which the elements of the periopticon have fused to form one single continuous neurospongium.

Fig. 33.—Diagram of the 'Acone' without any refracting bodies.

Fig. 34.—Diagram of the 'Pseudocone,' with the nucleus internal and the refracting bodies external.

Fig. 35.—Diagram of the 'Eucone,' with the nuclei external and the refracting bodies internal.

On a Peculiar Sense Organ in Scutigera coleoptrata, one of the Myriapoda.

By

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With Plate XVIII.

In the spring of the year I was fortunate enough to get a fair number of Scutigera in the South of Europe. In making an examination of their anatomy I found a sense organ which seemed to me to be of sufficient interest to render a more complete examination desirable. This organ is placed on the ventral surface of the head at a short distance behind the mouth and near the base of the mandibles. Its external appearance under a low power of the microscope (Zeiss's objective A A) is shown in fig. 1.

GENERAL FEATURES.

The organ which was first mentioned by Latzel, consists of a chitinous sac with a slit-like opening (fig. 1, *eo.*). The opening is placed between the base of the mandibles and the maxillæ. The sac has a somewhat complicated form which will be best understood by reference to four diagrams (see Plate, pp. A, B, C, D.

The first of these shows a rough outline of the appearance of the organ from the ventral side; the second, third, and fourth being diagrammatic sections through the dotted lines *AB, CD,* and *EF*. *B* is a transverse section through the anterior portion of the organ. It shows the main sac communicating with the

exterior by a narrow neck and two lateral recesses opening into the neck and placed ventrally to the main sac parallel with the ventral surface of the head. The section *C* taken through the median portion of the organ exhibits similar relations, excepting that the median dorsal wall of the sac is projected into the interior in two longitudinal folds which make a partial division of the sac into three portions, two deep, wide lateral pouches, and one deep narrow recess between them. This latter I shall speak of as the median recess. A third section, *D*, is taken through the hinder part of the organ. Here the lateral recesses and the slit-like opening to the exterior are absent and the two dorsal folds almost completely divide the main sac in three portions.

It is worthy of note that the effect of the median and lateral recesses is to produce a freely projecting lip or edge on the dorsal and ventral aspects of each pouch.

The general shape of the interior of the organ is therefore posteriorly that of two pouches projecting into the interior of the head, while between them is a median dorsal recess formed by the folds above described, which constitute the inner walls of the pouches where they approach one another. Anteriorly the division into two pouches is not so perfect, but there are two deep lateral ventral recesses. The slit-like opening to the exterior begins at the anterior end and extends about a third of the length of the whole organ.

The chitinous exoskeleton is continued into and lines the whole organ. It is not, however, of uniform thickness. In the median and lateral recesses and on the folds constituting the lips of the pouches it is smooth, but in the pouches themselves it is thrown into a number of folds and bears a large number of chitinous hairs (fig. 2, *h.*) which project into the lumen of the pouches. The folds form alternate ridges and depressions, so that when looked at from the surface through a microscope the chitinous lining of the pouches has a reticulated appearance (fig. 8). The hairs, whose length is about that of the diameter of each pouch, are of peculiar form (fig. 9). Each consists of a stout elliptical basal portion, the inner end of which is inserted

into the chitinous lining of the pouch and indeed projects for a short distance on the inner side of the latter.   The outer end is prolonged into a long fine hair.

General Features of the Internal Anatomy.

The hypodermic layer of cells or matrix lying beneath the exoskeleton accompanies the chitin round the lateral recesses, and at the edge of the folds which form the lateral lips of the two pouches becomes continuous with a thick layer of sensory epithelium which lines the greater part of the two pouches (fig. 2, *s. e.*).   On reaching the dorsal lips of the pouches, which lips bound laterally the median recess, the epithelium loses its sensory character and again becomes simple hypodermis.   On the dorsal part of the median recess the epithelium again becomes sensory in character.   The nerve supply is furnished by two short thick nerves (fig. 2, *N.*) which arise from the front portion of the suboesophageal ganglion.   The two nerves enter the sensory epithelium, one to each pouch, near the posterior part of the organ, and there breaks up into a number of fibres which become lost in the epithelium.   The form of the organ as indicated by the division into two pouches (fig. 2, *p.*) and the double nerve supply seem to me to show conclusively that it is double, and that each of the two pouches with its other parts is to be regarded as constituting a separate sense organ.

Histology.

The histology of the cellular tissues demands a more detailed account.   The cells forming the matrix from which the exoskeleton is renewed after each moult are large, rather columnar in their character, and have a well-defined nucleus. They are closely applied to the chitin and accompany it up to the end of the lateral recesses (refer to fig. 3, *hy.*) where the folds forming the ventral lips of the pouches begin.   Here the chitin is thrown into folds somewhat like those which characterise the surface of the pouches.   The hypodermic cells here lose their regularity of outline and follow the chitin into the

folds and irregularities into which it is thrown (fig. 3, *hy.*). Their nucleus is larger and stains more deeply. At the lateral ventral lip (fig. 2, *lvl.*) the cells are more elongated and more closely packed together, and gradually take the character of the sensory epithelium which forms the greater part of the lining of the pouches. These sensory cells are long and columnar and at their outer ends are prolonged into a blunt projection of less diameter than the rest of the cell (fig. 7, *oe.*) and about one third the length of the whole. At the folds which bound the median recess the cells lose their sensory character and take the form of the ordinary hypodermic cells. The mass of sensory cells at the top of the median recess which are continuous with the hypodermic cells are of a character distinct from those described as lining the pouches. They are of irregular elongated shape and resemble ganglion-cells, the inner end being sometimes bifurcated (fig. 6, *Bi.*). The sensory epithelial layer is of considerable thickness (fig. 2).

I have hitherto spoken of the epithelial layers simply as investing the chitinous pouches with their hairs, but I will now consider the means by which the hairs and cells come into relation. There is no doubt that the terminal parts of the sense cells project into the depressions (fig. 3) in the chitin, caused by the folds spoken of above, and that each chitinous hair is inserted into the chitin immediately outside this projecting part of a sense cell. I am also inclined to believe, though, owing to the small amount of material at my command my evidence on this point is not conclusive, that the bases of the chitinous hairs, i. e. the part which projects on the inner side of the chitinous lining, have a small cavity in their basal parts, into which a threadlike prolongation of the sense cell projects.

I have invariably found foreign bodies in the median and lateral recesses, and as the latter are in communication with the exterior they may possibly be grains of dirt or sand, but I think that they may be concretions.

Conclusions.

The active predatory habits of this Myriapod and its power of swift locomotion would seem to render well-developed sense organs a necessity to it; in fact it has facetted eyes in place of the simple eye-spots of most Myriapods.

The organ above described must, I think, be included among the great number of widely dissimilar organs usually classed together as auditory, and may be compared to the tympanic organ of insects.

The auditory organs of insects have been investigated principally by v. Siebold ('Archiv für Naturg.,' 1844), Leydig ('Müller's Arch.,' 1855 and 1860), v. Hensen ('Zeitschr. f. wiss. Zool.,' tom. xvi, 1866), and v. Graber ('Deutschr. der K. Akad. der Wissensch.,' Wien., 1875). The tympanic organ of the Acridiidæ consists essentially of a tympanic membrane supported by a chitinous ring. Places in the tympanic membrane are thickened, so as to form solid chitinous pieces of peculiar form, the internal surface of which is covered with indentations in which the extreme ends of the sensory apparatus end (Fr. Leydig, 'Müller's Archiv,' 1855, p. 401). The auditory nerve spreads out on these chitinous pieces and forms a ganglion, from which fibres ending in peculiar sense cells are given off. A trachea lies close to the ganglion internally to it, and not unfrequently swells to a vesicle.

On comparing the organ of Scutigera with such an organ there is found to be a great similarity in the general plan. Each pouch in Scutigera represents the insect tympanum. In both cases we have a thick nerve breaking up into a number of sensory elements, which end in depressed spaces in the chitinous membrane. With regard to the chitin hairs which project through the chitin in Scutigera, I think it will be worth while to consider Hensen's investigations on the auditory rods of insects (Hörstrifte, v. Hensen, l. c.). He makes an interesting comparison between these structures and the auditory hairs of the crustacean auditory sac, and draws the con-

clusion that the two structures present a very great morphological resemblance. If his arguments hold good it seems to me permissible to compare the hairs of Scutigera to those in the auditory sac in Crustacea, and also to the auditory rods (Hörstifte) in insects.

There is one point, however, in which the organ of Scutigera differs greatly from the tympanic organ, viz. in the absence of a tracheal vesicle. I think it doubtful, however, whether this tracheal vesicle is an essential part of the insect auditory organ. The swelling of the tracheal trunk seems not to take place in all cases (Leydig, l. c.), and Hensen, in giving what he considers the most probable hypothesis as to the action of the tympanic organ, says: "Die tracheen schwingungen sind ohne Bedeutung.' Balfour, in his short account of the auditory organ of terrestrial insects ('Comp. Emb.,' ii, 423), does not mention the tracheal vesicle.

I have examined this sense organ of Scutigera, both by dissection and by means of sections. I found that the tissues were best preserved by a mixture of corrosive sublimate and acetic acid. The difficulty of cutting the chitin in sectioning was overcome by embedding in very hard paraffin.

My investigations were entirely carried on in the Cambridge Morphological Laboratory.[1]

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[1] Since forwarding this paper (November, 1884) to the editor of this Journal my attention has been drawn to a paper by Dr. Haase in Schneider's 'Zool. Beiträge,' 1884, upon "Schlundgerust und Maxillarorgan von Scutigera."

As I am on the point of leaving England on a long voyage it is now too late for me to make an extensive reference to this work, but I may add that in my opinion Dr. Haase's observations do not necessitate any alterations in the foregoing paper.

DESCRIPTION OF PLATE XVIII,

Illustrating Mr. F. G. Heathcote's Paper " On a Peculiar Sense
Organ in Scutigera coleoptrata, one of the Myria-
poda."

Letters used in all the Figures.

m. Mouth. *f.* Furrow in chitin. *e. o.* External opening of sense organ.
o. Sense organ. *mxl.* 2nd maxilla. *N.* Nerve. *s. e.* Sense epithelium.
h. Chitinous hairs. *hy.* Hypodermis. *p.* Pouch. *lr.* Lateral recess. *Mr.*
Median recess. *lvl.* Lateral ventral lip. *Mdl.* Median dorsal lip. *ch.*
Chitin. *Bi.* Bifurcated end of cell at top of median recess. *oe.* Outer
end of cell. *lh.* Base of chitinous hairs projecting into interior. *bp.*
Basal part of chitinous hair. *fh.* Free end of hair.

Fig. 1 was drawn for me by Mr. Chapman ; all other figures were drawn by
myself with the aid of Zeiss's camera lucida. Fig. 2 is combined from three
sections.

Fig. 1.—Ventral view of head of Scutigera coleoptrata. The sense
organ is seen through the chitin. *m.* Mouth. *f.* A furrow in the chitinous
exoskeleton, marking out two irregular areas just in front of the organ. *e o.*
Opening of organ to the exterior. *o.* Organ seen through the chitin. *oc.*
Eye. *mxl.* 2nd maxilla.

Fig. 2.—Transverse section through the organ, showing the nerve (*N.*), sense
epithelium (*se.*), chitinous hairs (*h.*), hypodermis (*hy.*), median recess (*Mr.*),
and lateral recesses (*br.*) ; also the two pouches (*p.*). (Zeiss's c c objective.)
Owing to the action of the reagents used the sensory epithelium has shrunk
away from the chitinous lining of the sac. *lvl.* Lateral ventral lip. *Mdl.*
Median dorsal lip.

Fig. 3.—Transverse section through the region of the lateral recess, show-
ing the transition from the hypodermis to the sensory epithelium. *hy.* Hypo-
dermic cells. *se.* Sense cells. *br.* Lateral recess. *ch.* Chitin. *h.* Chitin-
ous hairs projecting into the pouch. *lvl.* Lateral ventral lip of pouch.
(Zeiss's F objective.)

Fig. 4.—Transverse section, taken so as to show the hypodermic cells (*hy.*)
joining the sense cells (*se.*) in the region of the median recess. (Zeiss's F
objective.) *hy.* Hypodermic cells. *se.* Sense cells. *ch.* Chitin.

Fig. 5.—Transverse section through the anterior part of the organ, show-
ing the hypodermic cells becoming continuous with the sense cells (*se.*). The

section is in front of Fig. 4. (Zeiss's D objective.) *se*. Sense epithelium. *ch*. Chitinous lining of the organ. *hy*. Hypodermis.

Fig. 6.—Tailed cell from sense epithelium at top of median recess. *Bi*. Bifurcated end. *oe*. Outer end. (Zeiss's F objective.)

Fig. 7.—Sense cells from epithelium *(se.)*. *oe*. Outer end of cell. (Zeiss's F objective.)

Fig. 8.—Surface view of chitinous lining of pouch seen from the internal side, showing the projecting bases of the chitinous hairs. *bh*. Base of hair projecting through the chitin. (Zeiss's water immersion L objective.)

Fig. 9.—Chitinous hair under high power. (Zeiss's L water immersion.) *bp*. Basal part of hair. *Bh*. Part of the basal piece which projects through the chitinous lining of the pouch towards the interior. *fh*. The hair itself projecting into the interior of the pouch.
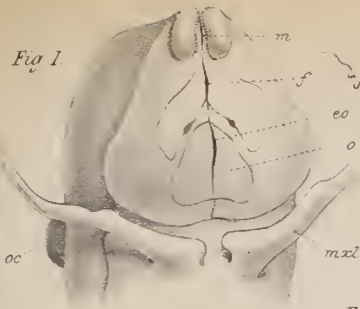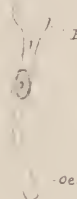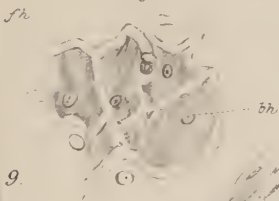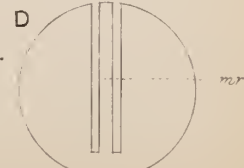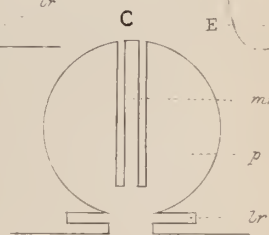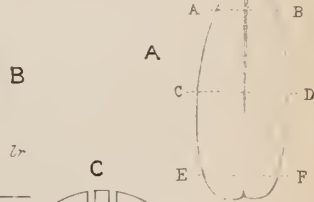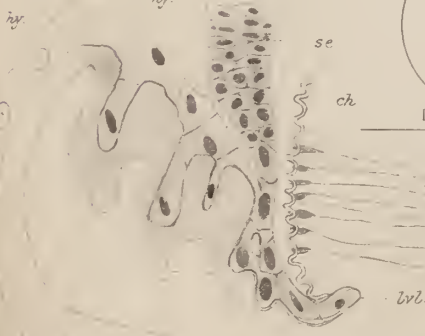
On the Structure and Development of Loxosoma.

By

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With Plates XIX, XX, XXI.

THE investigations which form the subject of the present paper were carried on during an occupation of the Cambridge University Table at Naples from January to July, 1884. An account of my main results was communicated to the Montreal meeting of the British Association in September, 1884, and a short abstract will appear in the forthcoming report.

PART. 1.—SPECIES OF LOXOSOMA FOUND AT NAPLES.

The specific characters of this genus have been found somewhat difficult to establish satisfactorily, and considerable doubt has on various occasions been thrown on the validity of many of the characters ascribed to the different species. Although the number of the tentacles and of the buds is not so constant as Schmidt (20) has supposed, the majority of the species which have been described are no doubt perfectly distinct from one another.

The following have come under my own observation at Naples :

1. L. Tethyæ, Salensky.—Number of tentacles in most cases twelve, but in many individuals thirteen or fourteen. Buds not

restricted to two, as supposed by Schmidt; they are formed
alternately on the two sides of the body, as in L. Kefer-
steinii (10), and a young bud usually occurs at the base of
every nearly mature bud, in addition to a third on the opposite
side, intermediate in age between the two just mentioned.
The stalk in most cases is at least twice as long as the calyx,
and is provided with a well-developed foot-gland with lateral
wing-like expansions. Round the free edge of the calyx, as
seen when the tentacles are retracted, is a definite series of
gland-cells described by Salensky (15). The ectoderm of the
stalk is arranged as eight longitudinal rows of cells (Pl. XIX,
fig. 9), and this is not the case in any of the other species
investigated.

Habitat, on the sponge Tethya.

2. L. pes, Schmidt (originally described by Schmidt as L.
singulare).—The tentacles are thick and short, and were ten
in number in all the individuals examined. In no case
observed was either side of the body provided with more than
a single bud. The stalk is usually considerably shorter than
the calyx; the foot-gland is very large, and the foot is pro-
vided with well-developed alate expansions. The character-
istic gland-cells of L. Tethyæ are not present, and the
ectodermic cells of the stalk are arranged irregularly. The
larva (Schmidt, No. 11, Taf. ii, fig. 25) resembles that of L.
Tethyæ.

Found in small numbers on Euspongia and Caco-
spongia.

3. L. singulare, Keferstein.—This species was found on
the ventral side of Aphrodite (as described by Barrois), and
of Hermione hystrix. The identification is somewhat
doubtful, Keferstein's individuals possessing ten tentacles,
whereas in the Naples species the number is larger, and in the
very few specimens obtained appeared to be twelve or thirteen.
The stalk, terminating in a slightly expanded disc, as in the
form described by Keferstein (2), is very short and is much less
distinctly marked off from the calyx than in the other species,
the whole animal being somewhat pear-shaped. None of the

individuals observed possessed more than a single bud, which was provided with a foot-gland, a structure absent in the adult. The larva (see Barrois' figure, No. 13) is not unlike that of L. neapolitanum (Kowalewsky, No. 3, fig. 10).

(There can be little doubt that the species just described is identical with L. claviforme, Hincks (26), occurring on Hermione hystrix. Hincks acknowledges that his diagnosis is very incomplete, owing to the bad preservation of his material, and it has seemed to me better, therefore, to identify my species provisionally as L. singulare, until the distinctness of L. claviforme can be more satisfactorily shown.)

4. L. crassicauda, Salensky, found in large numbers attached to the floor of a tank in the Zoological Station. The number of buds is large, two, three or more occurring in most cases on each side of the body. The number of tentacles is typically eighteen, but is by no means constant (fig. 1 ;—seventeen tentacles). The individuals are much larger than those of any other species discovered; the stalk is very long, with no regular arrangement of its ectoderm cells, the foot-gland being absent or (Schmidt) preserved as a rudiment in the adult; the termination of the stalk is cylindrical. Numerous gland-cells occur at the edge of the calyx, whilst the special development of two posterior sense organs (fig. 1, ps.) is a noteworthy feature of the species. The larva is at present unknown.

5. L. Leptoclini, Harmer, new species, not uncommon at Naples on the compound Ascidian Leptoclinum maculosum. I propose for the new form the specific name Leptoclini; as far as my observations extend, it is confined in its occurrence to the genus Leptoclinum, although I have searched for it on numerous genera of Sponges and Ascidians. The number of tentacles is perhaps invariably ten in the adult; the buds are few, although the occurrence of three at the same time, as in L. Tethyæ, is by no means rare. The individuals are small, their total length (with the stalk) averaging about half a millimetre (the measurements having been made from glycerine preparations). The stalk is at most about equal in length to the calyx, which has the character-

istic form represented in fig. 2. The foot-gland is well developed, the termination of the foot is alate, and there is no definite arrangement of the ectoderm cells of the stalk. At each side of the calyx occurs a series of gland-cells (fig. 2, *gc.*). The larva differs considerably in structure from any other Loxosoma larva at present described. Perhaps the most characteristic feature of the adult is the presence of a group of remarkable cells (fig. 5, *ac.*) at the apex of the vestibule. The stomach is divided into a median and two lateral portions, as represented in the figure.

Explanation of the terms Dorsal, Ventral, &c., as applied to descriptions of Loxosoma.

It will be convenient to define at once the use of such terms as " dorsal " and " ventral," " transverse," and so on. From reasons which will appear in the sequel, I am led to support the view that the ventral line of the body is that between mouth and anus, in opposition to Caldwell's theory (34) that this surface is dorsal in the Polyzoa.

The dorsal region is drawn out into the stalk on which the calyx or body of the animal is borne, whilst the anus is of course posterior to the mouth.

A transverse section is one which passes in the plane of the stalk through the right and left sides, and therefore parallel to the flat surfaces of the somewhat discoidal calyx, whilst a horizontal plane is at right angles to the long axis.

PART 2.—ANATOMY OF THE ADULT LOXOSOMA.

The entire animal consists of two parts, the calyx or body, and the stalk by which it is attached to its resting place. The two budding regions are situated at about the middle of the calyx, right and left; owing to the fact that the buds become free as soon as they reach maturity, Loxosoma, unlike almost all other Polyzoa, never forms colonies.

The ventral side of the body is produced into a free fold enclosing the vestibule on the oral face of the animal. The

tentacles, ciliated on their inner sides, are borne on the oblique lophophore, and can be extended to the exterior (fig. 1), or, when the animal is irritated, can be completely sheltered within the vestibule (fig. 2). In the latter case, the vestibular aperture is reduced by means of sphincter muscles to a small circular hole situated on the anterior side (fig. 2, *va.*).

The body is covered by a delicate, transparent cuticle, secreted by an ectoderm composed of a single layer of cells. The study of these cells is immensely facilitated by the use of nitrate of silver applied in a manner suggested to me by Dr. W. H. Ransom, of Nottingham, the essential feature of the process consisting in washing the tissues in a solution of a neutral salt (KNO_3) which gives no precipitate with nitrate of silver, the solution having the same specific gravity as sea water (*i. e.* about 5 per cent. KNO_3 in distilled water). By means of this process a precipitate of silver chloride can be completely avoided, whilst the tissues suffer practically none of those changes which are brought about by washing with distilled water (No. 44). Pl. XIX, fig. 3, represents the appearance of the posterior side of the calyx treated in the above manner, these preparations being in nearly all instances so successful that it was possible to draw, by means of a camera lucida, the outline of every ectodermic cell.

These cells are of the following kinds:

(i) Ordinary epithelial cells, which are large and polygonal, with a conspicuous nucleus (fig. 7); they occur over the whole of the calyx and stalk, on the outer and part of the inner surface of the vestibular fold, and on the dorsal and lateral portions of the tentacles. The inner sides of the latter are covered with a much higher epithelium, which is composed of cylindrical cells provided with large active cilia. In most regions of the ectoderm it is not possible to distinguish any definite arrangement of the epithelial cells, which, however, on the stalk of L. Tethyæ occur in eight definite longitudinal rows, of which three are represented in fig. 9.

(ii) S e n s e   C e l l s.—In fig. 3 are indicated, scattered at irregular intervals, certain small round areas, which occur

amongst the purely epithelial cells. These structures, much less numerous than the latter, are in reality ectoderm cells, which form the terminations of sensory nerves. Each is a nucleated cell of small size, bearing one or more fine, stiff, tactile hairs, projecting into the water (fig. 8).

(iii) Gland Cells.—In certain regions of the body occur large unicellular glands opening to the exterior, and presenting a considerable amount of variation in the different species. Attention was first directed to these structures by Salensky (15), who described them in L. crassicauda and L. Tethyæ. In silver nitrate preparations of the former they may be seen to open between the ordinary epithelial cells by wide apertures (fig. 7, *agc*), whose margins are surrounded by a thick black line : in most cases they occur in a row parallel to the edge of the vestibule, and reaching almost to the beginning of the stalk. The apertures of the gland-cells are situated just on the anterior side of the edge, in the condition of complete retraction of the tentacles. In L. crassicauda two very distinct forms of gland-cells are present (fig. 8) : the one nearly transparent and filled with large spheroidal vacuoles ($gc.^1$) ; the other opaque, and composed of a large number of small granules ($gc.^2$); the nuclei of the gland-cells can be detected in sections. In L. Leptoclini (fig. 2) the gland-cells occur only at the sides of the calyx, and differ from those of L. crassicauda in possessing a large central vacuole surrounded by a thin layer of protoplasm, embedded in which is the nucleus. Whether the characteristic apical cells of L. Leptoclini (fig. 2, *ac.*) belong to the same category as the gland-cells appears doubtful; it is, perhaps, more probable that they belong to the mesoderm, as in section they appear to be covered externally and internally by a layer of epithelium. The function of these curious cells has not been made out; their position is sufficiently indicated by fig. 2.

In L. Tethyæ the gland-cells are very numerous, and closely packed together round the ventral end of the vestibule; they are composed of a granular material, which stains with great

difficulty.   Salensky describes, in the stalk of L. Tethyæ and in that of L. crassicauda, definite longitudinal rows of gland-cells, which have altogether escaped my observation.

Large oval granular cells are found in the gelatinous tissue of the tentacles and other regions of the body of Pedicellina (fig. 5, *clp*), and have been already mentioned by Nitsche (6). In my preparations I can discover no aperture from these cells to the exterior, so that it is probable that they are not of the same nature as the gland-cells of Loxosoma.

Foot-Gland.—This structure is eminently characteristic of the genus Loxosoma, many species retaining it in the adult condition; whilst in others it is present only in the bud.   In the former case, when the long axis of the foot lies in a straight line with that of the stalk, the "sole," by which attachment takes place, is on the anterior side, the apex of the foot in its usual position being directed posteriorly.

In L. pes, L. Tethyæ, and L. Leptoclini (as well as in L. Raja and L. cochlear, Schmidt), the foot is provided with wing-like lateral outgrowths (fig. 2, *al.*).   Barrois (13) from the presence of these outgrowths, which cannot in reality be held to be distinctive of any single species, has given the name of Loxosoma alata to a species discovered by him, and states that it is identical with L. pes.   As there can be little doubt of the specific distinctness of L. pes, L. Tethyæ, and L. Leptoclini, all of which are provided with alate expansions of the foot, it is obvious that no sufficient diagnosis of L. alata has at present been given.

The foot-gland has been described by Schmidt (11) as consisting of a round granular mass situated in the angle of the foot, and communicating with a duct which traverses the "sole" and opens at the free end of the foot by a small pore; the duct is surrounded by four rows of large cells.   In L. neapolitanum, Kowalewsky (3) states that the duct opens by a series of pores occurring in variable number along the whole of its course.   In L. pes, L. Leptoclini, and L. Tethyæ, my observations confirm neither of these statements; and it is worthy of remark that in L. pes I have had occasion to

examine the same species whose foot-gland has formed the subject of Schmidt's description.

The foot-gland appears to me to be composed of two distinct portions ;—(i) the " gland," and (ii) the " duct " of Schmidt. The " gland " consists of a small number of granular nucleated cells arranged round a central lumen, as described by Kowalewsky. (See fig. 21, a section passing somewhat obliquely through the foot, so as to avoid the " duct.") This glandular mass opens at the angle of the foot by a pore which has no connection with the " duct " (fig. 2). The latter in the three species I have examined is in reality an open groove (fig. 20), extending along the whole of the " sole " of the foot, as may be clearly seen by means of sections or of glycerine preparations. The rows of cells described by Schmidt along the " duct " are the high ectoderm cells lining the open groove. In certain conditions, when its edges are closely approximated, the appearance of a closed duct is produced. The foot-gland originates as a longitudinal groove on the anterior side of the base of the bud, which is attached to the adult by that portion which will ultimately become the free end of the foot. During the period when I had fresh Loxosoma at my disposal, I failed to give any special attention to the development of the " gland," but (from glycerine preparations) it appears to me that the longitudinal groove, soon after its formation, becomes constricted into two parts (fig. 19)—a longer dorsal and a shorter ventral portion. The latter forms the " gland " after becoming completely separated from the elongated portion of the groove, which persists as such in the adult.

Nervous System.—The character of the nervous system of Loxosoma has always been exceedingly doubtful, although from the analogy of Pedicellina there could never be any real question of the existence of a ganglion between mouth and anus in the former. Such a structure has, however, never been correctly described, Salensky's identification being, as I shall attempt to show, erroneous. As a matter of fact, the ganglion has been unmistakeably figured by Nitsche (10), Schmidt (11), and Salensky (15), but by all these observers has

been considered to form a part of the generative apparatus. According to Nitsche, there exists in the bud, lying transversely across the intestine, on its oral side, a dumb-bell shaped mass (l. c., Taf. xxv, fig 19, *GA*) which Nitsche does "not hesitate to identify as the rudiment of the generative organs." The same structure may be observed in the adult, and is represented in (Nitsche's) Taf. xxv, fig. 5, *GA*. This "generative rudiment" has been figured by Schmidt as the "testes" in L. Raja[1] (Taf. i, fig. 1, *t*), whilst Salensky, in his fig. 3 of Pl. xii, has lettered the same organ *gs.*, without explaining the meaning of these letters; from the text, however, it seems obvious that the structure in question is considered either a portion of the generative system, or some other form of gland. I have not the slightest doubt that the organ so consistently described by the above observers as a gonad is in reality the suboesophageal ganglion. The structure figured by Nitsche (No. 10, Taf. xxv, fig. 5, *N*), and considered by him to be possibly nervous, I believe to have no existence as a special organ. Schmidt (20) has expressed his opinion that Salensky's "ganglion" is really the empty vesicula seminalis, and with this view I entirely concur. Salensky has pointed out the difficulty of discovering the "ganglion," except in young individuals which have developed no generative organs: in the adult one may assume that he recognised the true nature of the vesicula seminalis from the presence of the spermatozoa. Although Schmidt is right in denying the nervous nature of this supposed ganglion, his opinion that the large nerves figured by Salensky are also parts of the generative apparatus is erroneous; the existence of these nerves cannot be

[1] On p. 7 of No. 11, Schmidt says, " Ich finde bei Loxosoma singulare oberhalb der Hoden, aber auch zwischen Oesophagus und Enddarm quer gelagert eine Art von Nervenband oder Doppelganglion, dessen Bedeutung mir aber desshalb sehr fraglich, weil ich bei den anderen Arten nichts Entsprechendes gesehen." Schmidt has thus apparently really identified the ganglion in L. singulare, although, led astray by the belief that the same organ was a gonad in L. Raja and other species, he has failed to convince himself of the correctness of his own identification of the organ in L. singulare.

doubted, and Salensky's account of them is quite accurate.
The latter observer has figured sensory hairs on the tentacles
of an advanced bud of L. crassicauda, but has fallen into a
singular error in denying their existence in the adult, as in
this very species the sense hairs are undoubtedly well developed
in the mature animal.

According to my own observations, the nervous system has
the following characters (Pl. XIX, fig. 1). The dumb-bell
shaped ganglion (*ga.*) lies transversely across the intestine,
immediately on its anterior or oral side (fig. 11). In sections
of L. Tethyæ (fig. 16) it is seen that the organ consists
medianly of a fibrous commissural portion in which there are
no ganglion cells, and of two lateral ganglia, in each of which
the cells form a layer round the outer end of the commissure.
In these preparations it is not possible to distinguish the
outlines of the ganglion cells, the number of which is, how-
ever, clearly indicated by the nuclei. There is no doubt that
the central portion of the ganglion is really fibrous, as above
described; and there is no trace whatever of a central duct,
whose presence is required on the hypothesis of the generative
nature of the organ. The ganglion can be easily observed in
any individual of all the species I have examined, and is found
not only in the buds but also in the adults, whether the latter
are provided with generative organs, male or female, or are
sexually immature : — It can be discovered in sections as
readily as in the living animal.

The peripheral nervous system is most easily examined in
the living condition, L. crassicauda, on account of its great
transparency, forming a most favorable species for investiga-
tion. The most conspicuous part of the peripheral nervous
system is formed by a pair of tactile prominences on the
posterior wall of the calyx (already described by Vogt (12) and
Salensky (15) in other species), and by the strong ganglionated
nerves connected with these organs. From each end of the
ganglion passes off a strong nerve which swells into an en-
largement formed of bipolar cells (fig. 1, *gas.*). From the
end of the latter a nerve passes, without branching, to the

tactile prominence on either side of the posterior wall of the calyx, situated not far from the level of the ventral surface of the stomach (fig. 1, *ps.*).   Each of these sense organs, whose structure is described below, bears a tuft of long stiff hairs. The tentacles are provided with a rich development of sense-cells, supplied by nerves (one for each tentacle) connected with the ganglion.   Owing to the somewhat varying number of the tentacles, the arrangement of their nerves cannot be absolutely constant, although certain general features can always be made out.   The tentacles belonging to the posterior half of the lophophore appear to be invariably supplied by two pairs of nerves running in the posterior vestibular wall.   The more median pair arises directly from the ganglion, each of the two nerves usually supplying three tentacles in the manner indicated in the figure.   The outer pair arises from the nerves of the posterior tactile prominences, and usually forks so as to give rise to two tentacular branches.   The origin of the nerves belonging to the tentacles of the anterior half of the lopho-phore is less easy to determine; but their arrangement, as given in fig. 1, is probably not very inaccurate.   With the exception of the two pairs figured, no nerves were with certainty observed to arise directly from the ganglion,[1] although it is possible that those supplying the dorsal regions of the body may have this origin.   At the base of each tentacle the nerve swells into a small ganglion (fig. 1, *tga.*), consisting in most cases of about four or five bipolar cells.   This ganglion gives off one or two nerve-fibrils, passing directly to as many sense-cells, and is prolonged into a fine nerve, which passes up the axis of the tentacle, the rest of whose sense-cells it supplies. These tactile organs are not very numerous, and they occur entirely on the outer (unciliated) and lateral portions of the tentacle.   Each bears a fine stiff hair, or more rarely two or three.   A single large hair occurs in the middle of the convex side of each tentacle near its apex, and when these organs

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[1] In the adult, there is no trace of a brain or of circumœsophageal com-missures, even the tentacles of the anterior portion of the lophophore being undoubtedly supplied with nerves from the subœsophageal ganglion.

are retracted the hairs radiate towards the centre of the vestibular orifice, immediately behind which they form a circlet, so that nothing of an irritant nature can pass through the aperture without at once coming into contact with one or more of the tentacular sense hairs.

The calyx, like the tentacles, is well supplied with sense hairs, which are most numerous in the neighbourhood of its edge; they occur (rarely) on the ventral portions of the stalk, although they are absent nearer its attached end. The connection of the more dorsal sense-cells with the ganglion could not be made out, in consequence of the opacity of the stomach. On the right side of fig. 1 is represented a nerve passing down the stalk, and giving off a branch to a sense-cell. In other regions of the body sense-cells are supplied from various parts of the nerves; none were discovered with certainty on any part of the inner wall of the vestibule, although the elongated cells of the epistome and oral end of the œsophagus have doubtless a sensory function, being probably endowed with the faculty of taste or smell.

The most reliable means of noticing the distribution of the sense-cells is in silver-nitrate preparations, fig. 3, for instance, representing the entire posterior wall of the calyx of L. crassicauda. In addition to the large epithelial cells may be observed very small cells which are provided with sense hairs in the living condition. By staining the silver preparations with picrocarmine (fig. 7), each of the small areas of fig. 3 was seen to be provided with a nucleus, the whole structure thus forming a small ectoderm cell.

In picrocarmine-silver-nitrate preparations was further observed the direct continuity between the latter and a nerve fibril, swelling into a bipolar ganglion-cell, whose deeper process passed directly into a nerve (see also figs. 1 and 8). This connection of the sense-cell with the central nervous system by means of the interposition of a ganglion-cell invariably occurs, whether the latter is isolated in the gelatinous tissue of the body, or is a constituent of the ganglion at the base of a tentacle (fig. 1, *tga*.). The connection of these cells with the

ectodermic sense-cells may be seen either in the living animal or in glycerine preparations in a manner which leaves room for no doubt as to the actual existence of this arrangement.

It remains now to consider the structure of the two posterior sense organs of L. crassicauda. In silver preparations (fig. 4) each is seen to consist merely of an exaggerated sense-cell, bearing a number of hairs, and provided with a large nucleus. The sense-cell passes into a nerve continued into a large number of ganglion-cells, instead of one only, as in other regions of the body of this species. The increase in the number of these cells is no doubt correlated with the speciali-sation of the sense organ. The arrangement in L. pes indi-cated in fig. 6, forms an intermediate condition between the two kinds of sense-cells of L. crassicauda. The posterior sense organs of the latter differ from the sense-cells borne on other parts of the body in forming distinct papillæ, at the apex of each of which is an enlarged sense-cell. In most cases (fig. 6) three of the flat epidermic cells are concerned in the formation of the papilla.

In Pedicellina no special attention was given to the peripheral nervous system. The ganglion (ga.) is, however, shown in fig. 12, a horizontal section; it consists of a central fibrous mass and of peripheral ganglion-cells. Instead, however, of being greatly elongated transversely, as in Loxosoma, it is oval; at the sides come off several pairs of nerves.

In a picrocarmine-glycerine preparation of Pedicellina, each tentacle (fig. 5) is traversed by a fine nerve, which gives off branches on its outer side, each passing into a bipolar ganglion-cell connected with an ectodermic sense-cell; one of the latter appears to be provided with a sense hair.

In the larva of Pedicellina, Hatschek (14) has called attention to the existence of tactile hairs on various parts of the body (Taf. xxix, fig. 26). These have exactly the appear-ance of the sense hairs of the adult Loxosoma, but their connection with the nervous system has not been observed. To the same category probably belong the stiff hairs arranged in a

ring round the foot-gland or sucker of the larva of both P e d i-
c e l l i n a   and   L o x o s o m a.   Sensory   hairs   have   ben
described in various species of E c t o p r o c t a; Nitsche (4), for
instance, has figured on the tentacles of A l c y o n e l l a f u n-
g o s a, tactile hairs exactly like those of L o x o s o m a, and
arranged singly or in groups of two or three.   In the former
case each hair, and ɪn the latter each group of hairs, is no
doubt borne on a sense-cell similar to those of L o x o s o m a.

Sense organs, ɪn external appearance resembling the pair
described above in L. c r a s s i c a u d a, occur in R h a b d o-
p l e u r a[1].   Lankester has suggested the possible homology
between these organs and the "osphradia" (Spengel's olfactory
organ) of the M o l l u s c a.   Although this homology is not
impossible, the character of the tactile organs of L. c r a s s i-
c a u d a seems to indicate that the posterior sense organs are
merely specialised sense-cells, exaggerated in size, and I there-
fore regard it as probable that these organs in the P o l y z o a
have no homology with the osphradia of M o l l u s c a.

The only portion of the nervous system whose development
I have followed in the bud is the ganglion (in L o x o s o m a).
Although the share which is taken by the derivatives of the
different germinal layers in the budding processes of this genus
is still obscure, the ganglion may be definitely stated to origi-
nate from ectoderm cells.   It is, in, fact, developed from
the floor of the vestibular cavity, and this can only be regarded
as ectodermic, whatever may be the origin of the alimentary
canal.   In a longitudinal section through a fairly-advanced bud
(fig. 15) it is seen that a narrow slit-like diverticulum of the
vestibule passes behind the epistome.   This diverticulum,
which remains ɪn very much the same condition throughout
life (see fig. 11), does not give rise *in toto* to the ganglion, which
ɪs merely formed by a differentiation of some of its ectodermic
cells.   Hatschek (14), in his account of the budding of P e d i-
c e l l i n a, describes the development of the ganglion as a diver-

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[1] These organs are on the posterior side, and are two in number; their
position, however, differs somewhat from that of the similar organs of L.
crassicauda.

ticulum from the vestibule, at a later stage pinched off as a sac containing a small lumen (Taf. xxx, fig. 40, G). It appears to me, however, unlikely that Hatschek's account is perfectly correct. Fig. 12 is a horizontal section through a Pedicellina. The ganglion (*ga.*) is oval, and consists of a peripheral layer of cells, enclosing a central fibrous mass, as in Loxosoma. In order to transform Hatschek's ganglion rudiment into the adult condition it would be necessary to atrophy the lumen and to replace it by a development of nerve-fibres. The latter would thus be formed from the primitively external surface of the cells, a conclusion which appears most improbable, since the position in which nerve-fibres originated phylogenetically was no doubt the deeper surface of cells which formed part of the outer ectoderm. This consideration, as well as the history of the ganglion in Loxosoma, leads me to suspect that the lumen in Hatschek's ganglion is in reality the commencement of the fibrous tissue, which in optical sections might easily be supposed an empty space. Similarly Nitsche (10) has described the ganglion of Alcyonella as originating as a diverticulum from the tentacle sheath. I regard it as probable that the explanation which I have suggested for Pedicellina will hold also for Alcyonella.

Salensky has pointed out the similarity between the two posterior sense organs of L. crassicauda and the similar organs of Rotifera (*vide* Möbins, No. 9, Taf. v, fig. 2). These organs in Brachionus consist of a pair of bunches of fine hairs, borne laterally on the surface of the body, and each supplied by a nerve from the brain. Möbius describes, further, certain bipolar ganglion-cells passing by means of a nerve-fibril directly to the body-wall, although no tactile hairs are described in this position.

Alimentary Canal.—The general characters of this system are already well known, and a reference to the figures accompanying this paper will sufficiently explain its anatomy. Behind the mouth (fig. 11) is a large epistome, the size of which has hitherto been hardly sufficiently recognised, and in extent, indeed, is not far inferior to the buccal shield of

Rhabdopleura (its homologue). Its anterior portion, which forms the posterior boundary of the mouth, is composed of very long, ciliated, rod-like ectoderm-cells, with much elongated nuclei (figs. 11 and 18). The posterior wall of the epistome is formed of large cells, and bounds anteriorly a diverticulum from the vestibule. At the bottom of this diverticulum is the ganglion.

The œsophagus communicates with the dorsal and anterior end of the stomach, as indicated in fig. 2, the passage of the stomach into the intestine being shown in fig. 1 and fig. 11. The rectum is separated by a constriction from the intestine; in L. crassicauda it protrudes into the vestibule as a large cone-like process (fig. 11), whilst in most other species the posterior wall of the rectum is closely applied to that of the vestibule, so that in these cases there is no free rectal cone.

The whole of the alimentary canal in L. crassicauda is lined by cilia, even the "liver-cells" of the stomach forming no exception to this statement. These cells are nucleated peripherally, whilst more centrally they contain yellow "hepatic" spherules, which give their characteristic colour to this part of the stomach.

In L. Leptoclini the stomach is produced laterally into a pair of wing-like outgrowths (fig. 2). In some species the occurrence of a parasite, a holotrichous Infusorian, swimming about in considerable numbers in the stomach, is very constant; three of them are seen in fig. 11 (*I*).

Connective Tissue and Muscular System.—These structures are best studied in the living animal or in glycerine preparations. The whole space between the ectoderm and the alimentary canal is completely filled, either by the gonads and other organs, or by a gelatinous matrix enclosing connective-tissue-cells, muscle-cells, &c.

The Entoprocta possess no body-cavity nor any definite system of spaces representing this structure.[1] The gelatinous matrix is perfectly transparent and hyaline, and, I believe,

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[1] Paired spaces, shown in fig. 11, usually occur in the epistome of L. crassicauda.

corresponds completely to the substance existing in the same position in an ordinary Trochosphere before the appearance of a definitive body cavity.

The connective-tissue cells of Loxosoma are large and granular (fig. 10), usually elongated, and giving off processes anastomosing with those of other cells; they occur in all parts of the body—in the axis of the stalk, at the sides of the stomach, in the tentacles, and so on. In Pedicellina (fig. 5) these cells are more similar to ordinary stellate connective-tissue-corpuscles. The muscle-fibres are elongated nucleated spindle-shaped cells, often branched at their ends; they are best developed in the stalk, where they form a longitudinal layer immediately beneath the epidermis. Special muscular fibres are connected with the foot (in those cases where the foot-gland persists), the tentacles, and other parts of the body. I have nowhere observed with certainty the endings of nerves in the muscles.

Excretory Organs.—With the details of the structure of these organs, so important for a correct appreciation of the systematic position of the Entoprocta, we are almost altogether unacquainted. Schmidt (11) has alluded to the nephridia, but appears to have considered them as parts of the generative system. Hatschek (14), who has detected them in both adults and larvæ of Pedicellina, makes the important statement that in the former the nephridia are composed of perforated cells, whilst Joliet (24), who has devoted an entire paper to the treatment of these organs, asserts that their walls are so delicate that the existence of an excretory function is doubtful.

The nephridia of Loxosoma are found lying on the ventral wall of the stomach, one on each side of the œsophagus (see Joliet's figure), and situated near the anterior surface of the body. In examining their structure, best observed in the living condition, the animal should be looked at from in front. The proximal portion can be investigated with more ease than the part which is nearer to the external aperture, since the organ in the latter position is covered by the œsophagus,

through whose thick walls the examination of fine details is a
matter of great difficulty. The proximal part consists of a
small number of cells (fig. 17), which, in opposition to Joliet's
statement, I consider undoubtedly excretory in function. The
number of these cells seems to be invariably about four (L.
crassicauda); they are of a distinct yellowish-green tint, the
general appearance of which I have attempted to indicate in
the figure, although on examination with high powers it is
seen that this is entirely due to the presence of numerous
granules which alone are coloured. The distal portion of the
nephridium is a colourless duct, which presumably has no
excretory function. In this portion only two nuclei were
observed, the remainder of the duct being partly concealed by
the œsophagus, although its cilia could be distinctly made out
almost as far as the external opening (fig. 17). It seems to
me certain that the two nephridia open independently, their
apertures occurring in the depression of the vestibular floor
situated behind the epistome; there can be no doubt that
the nephridia open to the exterior in front of the
ganglion. The proximal portion is invariably curved, the
concave side being internal, and the entire organ is perforated
by a ciliated duct, beginning at its proximal end, and opening
distally into the vestibule. The proximal cell I believe to be a
flame-cell, as represented, although I am not prepared to state
positively that this is really the case. In many individuals,
however, I have felt satisfied that I have detected the single
flagellum of the flame-cell, the difficulty consisting in the dis-
tinction of the movement of a flagellum of this kind from the
appearances produced by the co-ordinated movements of a
series of cilia like those occurring in most parts of the nephri-
dial duct. The proximal portion of the duct, which ends
apparently cæcally, is somewhat swollen, whilst the next
portion is constricted; at the bend of the nephridium, the duct
increases very largely in calibre, the widened portion being
continued slightly beyond the termination of the excretory
cells, and thence narrowing to the aperture. The duct per-
forates the individual cells of the nephridium, the cell limits

being indicated on the exterior by constrictions, whilst in glycerine preparations made with osmic acid and picrocarmine it has been possible to observe the nuclei of the perforated cells, seen also with less certainty in the living condition. My observations on the nephridia of Loxosoma are confined entirely to the adult, but from the analogy of Pedicellina there can be little doubt that the larva also is provided with an excretory organ.

Although the nephridium described above differs in a very marked degree from that of the Brachiopoda, it has the closest possible similarity to the head-kidney of many Trocho-spheres (see Hatschek's papers on Polygordius (17) and Echiurus (25). The resemblance between the nephridium of Loxosoma and the head-kidney of the larval Polygordiu is, however, more striking than could have been anticipated, since Fraipont (43) has shown that each branch of the head-kidney of the larval Polygordius ends blindly, and has not in reality the form of a ciliated funnel opening into the body cavity. The probability that the excretory organ of the adult Loxosoma is to be regarded definitely as a head-kidney is, however, most clearly shown by an inspection of the results of the researches of Eduard Meyer on the head-kidneys of various Annelid larvæ. Dr. Meyer was kind enough, during my stay at Naples, to show me his drawings (at present unpublished) of these structures, which resemble the nephridia of Loxo-soma in a more striking manner than does even that of Polygordius, the similarity being obvious in the number of the excretory cells, in the relative size of the lumen in different parts of the organ, in the mode of termination in a flame-cell, and in other points.

The Trochospheral head-kidney opens to the exterior on each side in front of the anterior end of the ventral nerve-cord, as is the case in the Echiurus larva (Hatschek), in the adult Loxosoma, and (Joliet) in the adult Pedicellina. Al-though it is by no means impossible that fine apertures may really exist in the proximal end of the head-kidney of Loxo-soma, it is probable that the flame-cell termination, situated

in the "primary body cavity," is morphologically different from the ciliated funnel which opens into the "secondary body cavity" in Chætopoda, Mollusca, and Brachiopoda. This is the view adopted by Lang (47), who has pointed out (p. 678) the support afforded to it of Ed. Meyer's observation that the ciliated funnel in Polymnia (Terebella) is in its first development quite separate from the excretory organ, with which it secondarily enters into connection. It appears to me, therefore, that the Polyzoa have remained (in this respect at any rate) in a far more archaic condition than the Brachiopoda, which possess a nephridium provided with a ciliated funnel.

It is probable, as Joliet (24) has already pointed out, that the "segmental organ" which has been described in some Ectoprocta is not the homologue of the nephridia of the Entoprocta.

I have in no species of Loxosoma succeeded in discovering the paired organs described by Salensky (15), and supposed by him to be renal. They are said to occur at the sides of the intestine in L. crassicauda, each consisting of a group of eight stalked cells opening to the exterior by a common duct.

Generative Organs.—The gonads of the Entoprocta are undoubtedly "idiodinic," to adopt Lankester's term (No. 38, p. 682), *i.e.* they have their own ducts to the exterior, and do not make use of nephridia for the extrusion of the generative products. The Entoprocta have usually been described as hermaphrodite, although some observers, like Kowalewsky (3), and Vogt (12), have asserted the separation of the sexes. Although I have examined a very large number of specimens of Pedicellina and Loxosoma, belonging to various species, I have in no single case been able to find in the same individual mature ovaries and testes. One or two of my preparations perhaps indicate that male and female generative organs can be developed in the same individual at different seasons; and I am inclined to believe that this is what really happens.[1]   In

[1] The fact that buds are developed indifferently on males and females alike perhaps indicates the correctness of this view.

L. pes the structure of the generative organs is certainly
more complicated than in most of the other species, in which
I have invariably found that mature ovaries and testes are
mutually exclusive. It is easily shown that individuals contain-
ing developing embryos in their vestibule are not provided with
testes in the species of Loxosoma and Pedicellina which I
have examined. In some cases (as in fig. 16) a vesicula seminalis
containing spermatozoa is found, although the testes seem to
be completely absent. This fact, perhaps, indicates that the
male gonads, which must have been originally present, have
atrophied in order to make room for the development of the
ovaries.

L. Tethyæ and L. Leptoclini.—The generative organs,
in individuals of either sex, consist of a pair of glands situated
at the sides of the body in the region marked *ot.* in fig. 2;
they lie on the ventral side of the stomach. In the male
(fig. 13) the testes are large bodies, consisting of a capsule
enclosing a mass of sperm mother-cells and mature sperma-
tozoa (with intermediate stages). I have not studied the
details of the spermatogenesis. From each testis runs towards
the middle line a short duct (*vd.*), opening into the posterior
side of a large oval vesicula seminalis, which in mature males
always contains numbers of elongated filiform spermatozoa,
which execute various movements even before their escape to
the exterior. The aperture of the vesicula into the vestibule
is very difficult to discover, but has apparently been seen by
Vogt, although this observer's statements have been most
unjustly questioned by Schmidt. It is worthy of note that
the ganglion can easily be observed in all mature males, and
that Schmidt's identification of this structure as the "testes"
is hence undoubtedly erroneous.

In the female the gonads have the same position as in the
male, and open by a single pore into the space between the
epistome and the ganglion (fig. 14). This pore (*p.*) leads into
an unpaired oval cavity, from which proceeds on each side a
duct to the ovary. The arrangement in Pedicellina is very
similar, except for the fact that the unpaired portion is pro-

longed into a passage opening at the end of a projecting papilla.
In this genus, however, the ovaries are situated between the
ganglion and the intestine, whereas in Loxosoma they are
found between the ganglion and the œsophagus, as indicated
by the position of the unpaired part of the generative duct.
(Compare fig. 12 with fig. 14; notice also fig. 16, a male.)
The ovary in L. Tethyæ consists of a small number of cells,
of which only one becomes mature at the same time (fig. 14).
The cell which is becoming an ovum increases in size at the
expense of the neighbouring ovarian cells; the latter are
absorbed by the egg, or rather appear to fuse with it, so
that the nearly mature ovum contains several nuclei whose
cells are separated from one another only by indistinct
boundaries.   These disappear in later stages, and the absorp-
tion of other primordial ova by the developing ovum is then
only indicated by the fact that the latter is polynuclear.   At a
still later stage presumably, the nuclei of the nutritive cells
are absorbed, as the mature ovarian ovum contains only a
single nucleus.   In addition to this process of the fusion
of several ovarian cells to form a single ovum, another
method by which the latter is nourished takes place in
L. Tethyæ.   In many of my sections the developing ovum is
seen to be engaged in devouring curious masses marked vt. in
fig. 14, and differing entirely in appearance from the ovarian
cells.   The latter stain readily with hæmatoxylin, whilst
the bodies vt. hardly absorb any of this colouring matter;
they have in fact precisely the same appearance as the yolk
material diffused through the mature ovum and segmentation
spheres of this species of Loxosoma.   There can hence be
very little doubt that the bodies which are thus devoured by
the ovum play the part of a vitellarium.   In the ovaries of
L. Tethyæ I have seen no structures with any resemblance
to the bodies in question, whilst the gland-cells occurring
round the edge of the calyx (described by Salensky, vide his
figure) behave in exactly the same manner with respect to
colouring matters, and have the same characteristic granular
appearance as the bodies vt. in fig. 14.   These vitelline masses

in the ovum do not seem to be provided with a nucleus, although there can be little doubt that they are really of cellular nature, whilst the "gland-cells" of L. Tethyæ are usually true nucleated cells.   In spite of this difference, I can see no course open but to suppose that these "gland-cells" lose their nuclei, and are absorbed by the ova in the manner indicated by the figure.   If this is really the case, it is difficult to believe that the bodies in question really have the nature of gland-cells, and it is possible that they may belong to the mesoderm, and have no connection with the exterior.[1]

Whatever the origin of the vitelline masses observed in the nearly ripe ovum, it is a fact of some interest that the yolk in L. Tethyæ appears to be prepared in part by cells which have no connection with the ovary, and that the vitellarium is probably not a modification of a primordial germinal mass containing potentially both yolk-gland and ovary.   In L. Leptoclini, the only other species which I have found sexually mature at Naples in considerable quantities, I have not observed any processes for the nutrition of the ovum comparable to either of the methods described in L. Tethyæ; and it is a noteworthy fact that the "gland-cells" of this species differ in their histological character from any cells occurring in the other species which I have examined.

It is difficult to reconcile some of the statements of Schmidt with the appearance of my own preparations of the generative organs of the Entoprocta.   I have been unfortunately unable to study in detail the structure of the generative organs of L. pes, the form specially investigated by Schmidt (11 and 20), and I have reason to believe that these generative organs do

[1] In position, the "gland cells" of L. Tethyæ correspond with the " apical cells " (ac., fig. 2) of L. Leptoclini, except for their greater lateral extension (nearly as far as the middle of the calyx on each side) in the former species.   It appears to me not improbable that these two sets of structures are homologous, although I have in L. Leptoclini no evidence as to their nature.   Perhaps in this species the cells, originally playing the same part as those of L. Tethyæ, are at present mere functionless rudiments.

really differ from those of both L. Tethyæ and L. Leptoclini.
In Taf. i, fig. 1 (No. 11), Schmidt has figured the ganglion
of L. Raja as "testes," and what are doubtless the testes as
"ovaries;" so that his statements as to the hermaphrodite
nature of this species, at any rate, are based on a misunder-
standing. As may be concluded from the description of L.
pes, on p. 7, Schmidt seems further to have believed that the
nephridia formed parts of the generative organs. In Taf. ii,
fig. 8, he has represented (L. pes) a duct passing from the
vesicula seminalis to the "ovary" on each side, this "ovary"
consisting of a large rounded body containing "ova" and sper-
matozoa. From his second paper (No. 20) one may conclude
that Schmidt considers it probable that the spermatozoa cannot
escape to the exterior, their only exit from the vesicula semi-
nalis being through the ducts leading to the "ovaries." I am
convinced that Schmidt's "ovaries" are in reality the testes, and
his "ova" the sperm mother-cells. By referring to my own
fig. 13 it will be seen that the testes of L. Tethyæ have the
same characters as Schmidt's so-called "ovaries" in L. pes. I
must most strongly express my conviction that in no species of
Loxosoma is there any duct passing from the vesicula semi-
nalis to the ovaries, and that where such an arrangement has
been described the duct is really the vas deferens, and the
"ovary" is the testis. Vogt's account of the passage of a
bundle of spermatozoa from the vesicula seminalis to the exte-
rior is doubtless correct, although Schmidt has questioned its
accuracy. I have not observed the process of fertilisation in
Loxosoma.

What may be the nature of the bodies t and t' in Schmidt's
Taf. ii, fig. 8 (No. 11), I cannot say, but I am unable to agree
even with his second account of their character (No. 20). In
this paper he states that the dorsal one is a bud rudiment,
which is the same thing as the "generative rudiment" described
by himself, Nitsche, and others in the bud (that is to say, the
organ which on my view is the ganglion). The "bud rudi-
ment" described by Schmidt in the adult is thus very different
in nature from the structure identified by him as the same

organ in the bud; and it appears to me to have no real con-
nection with the budding processes.

In L. crassicauda, L. Leptoclini and L. Tethyæ there
is certainly no such " Knospenstock" as that described by
Schmidt. In the woodcut fig. 2, on p. 75 of Schmidt's second
paper the generative organs of a male L. pes have probably
been represented; *o* is in this case the testis, and the vas
deferens is not indicated. Schmidt's attempt to elucidate the
nature of the two pairs of bodies described by him respectively
as " testes" and " bud-rudiments" can thus hardly be con-
sidered successful, and I am unable myself to throw any fresh
light on the nature of these problematical organs of L. pes.

PART 3.—THE DEVELOPMENT OF LOXOSOMA.

Our present knowledge of the embryology of Loxosoma
is exceedingly limited, in spite of the statements of Vogt,
Barrois, and others on the subject. That of Pedicellina
has, however, been most accurately worked out by Hatschek
(14), whose account I can confirm in its main features, with
the exception of that part which refers to the development and
the nature of the " dorsal organ," his " Entodermsäckchen."
My study of the development of Loxosoma has been made
almost entirely by means of sections passing through the
embryos in various planes.[1] L. Leptoclini differs from
L. Tethyæ in possessing two specialised diverticula of the
posterior portion of the vestibule, one on each side of the
intestine, which by its projection as a large longitudinal ridge
(covered of course by ectoderm) into the vestibular cavity,
gives rise to the two diverticula. In these the earlier stages of
development take place, the older embryos, however, escaping
into the main vestibular cavity, and no doubt nourishing them-
selves on food particles brought to the vestibule by the cilia

[1] The material was preserved with osmic acid, picric acid, or corrosive
sublimate; the best staining of the embryos was obtained, after cutting, by
means of Mayer's method for fixing sections on the slide (39). In this case,
hæmatoxylin, picrocarmine, or picrocarmine followed by hæmatoxylin gave
the best results.

of the maternal tentacles, as is the case, according to Hatschek,
in Pedicellina. In L. Tethyæ there are no specialised
brood pouches, and development takes place in the general
cavity of the vestibule. In L. Leptoclini, however, where
the amount of yolk is very small, the lining of the two brood
pouches at the sides of the intestine consists of an epithelium
of high columnar cells, altogether unlike the flat cells which
line the greater part of the vestibule. During the earlier
processes of development (figs. 25—34) the embryo hardly
increases at all in size, if allowance is made for the appearance
of internal cavities (blastocœl, archenteron), by which the cell
layers are forced asunder.

At about the stage of fig. 43, however, the epiblast in certain
regions becomes very thin, and these portions are found closely
applied to the columnar epithelium lining the brood pouches
(fig. 47). The embryo forthwith commences to grow rapidly
(figs. 43—45), and although this increase in size may be partly
due to the digestion of food particles taken in by the mouth
(which is already present), it is probable that it is mainly due
to the activity of the columnar cells of the brood pouch.
These may be supposed to secrete a nutrient material through
the thin walls of the embryo into its primary body cavity,
which at this stage is a wide space between epiblast and hypo-
blast. The connection between the thin walls of the embryo
and the epithelium of the brood pouch is often so intimate that
careful observation is necessary to discover the existence of the
former (see fig. 47). It is probable, therefore, that in L. Lepto-
clini the embryos are nourished by a placenta composed of a
layer of glandular cells, modified epidermic elements of the
vestibule, forming a portion of the outer limit of the gela-
tinous substance (of the adult), which serves as a medium for
the diffusion of the various products of metabolism to the
different organs.

In Pedicellina echinata the epithelium lining the brood
pouch is similar to that found in the corresponding position in
L. Leptoclini. It is, however, thrown into numerous folds
(fig. 12), whilst no intimate connection is set up between the

embryos and the vestibular epithelium. It is hence probably the case that in Pedicellina the brood pouch can be almost completely shut off from the rest of the vestibule, nutrient substances being secreted into it by its glandular epithelium, which is largely increased in area by the formation of folds. This is, probably, in the main a consequence of the large number of the embryos present at the same time, and of the great increase in size which they experience during their stay in the brood pouch.

In the whole course of the development of L. Tethyæ, the increase in size of the embryo is small compared with that of L. Leptoclini and of Pedicellina; so that whereas the ovum of L. Tethyæ is much larger than that of L. Leptoclini, this difference in size being still very obvious in comparing, for instance, fig. 29 with fig. 60, or fig. 38 with fig. 62 (representing corresponding stages of the two species magnified to the same extent), the embryo of L. Leptoclini early equals that of L. Tethyæ in size, the free larva of the former species being considerably larger than that of the latter.

It is worth noting that the marked growth of the embryos of L. Leptoclini and of Pedicellina cannot probably be entirely ascribed to the fact that they devour particles of solid food, since in L. Tethyæ, whose embryo is quite early provided with a mouth, the increase in size during development is comparatively small.

Segmentation, Formation of the Germinal Layers, and Completion of the Alimentary Canal; Mesoblast.

L. Leptoclini.—The ovum (fig. 25), on escaping into the vestibule, segments completely, at first into two (fig. 26), and then into four (fig. 27). The latter figure was drawn in the living condition, and shows the vitelline membrane present at this and some of the later stages. I have not observed polar bodies, this being probably a result of my almost exclusive use of the section method. At the next stage represented (fig. 28), the embryo is a solid mass consisting of about eight cells,

four of which occur in the section; whilst in fig. 29 the number of cells is larger, and a segmentation cavity or blastocœl has made its appearance. The embryo at this stage is usually much flattened, partly owing to the pressure of larger embryos which are found at the same time in the brood pouch. The number of embryos in each brood pouch is, however, considerably smaller than in Pedicellina, and seldom exceeds about two or three. In fig. 30 it is seen that a slight depression has been formed in the centre of the flattened side, representing the commencement of the archenteron. The cells on the side of the blastopore are somewhat higher than on the opposite side, whilst the blastocœl is almost obliterated, owing to the pressure of other embryos. In fig. 31 the blastocœl is larger, although the invagination has proceeded a stage further. Fig. 32 and fig. 33 represent sections of other embryos slightly older than fig. 31. In fig. 33 the section has just missed the blastopore, so that the invagination appears solid, whereas in most other cases, a central depression is present from the first in the invaginating mass of cells. Fig. 32 shows the appearance of a large cell in the immediate neighbourhood of the blastopore; this is one of the two pole-cells of the mesoblast.

The figures 60 and 61 represent two early stages of L. Tethyæ, drawn with the same magnifying power as those of L. Leptoclini. In fig. 60 the blastocœl has just made its appearance. In fig. 61 the invagination has progressed to a considerable extent, the section passing transversely through the archenteron and blastopore, and cutting both of the pole-cells, which even at this early stage are completely shut out from any share in bounding the archenteron; they have already taken up their definitive position between the epiblast and the hypoblast.

Fig. 34 represents a section of a gastrula of L. Leptoclini; the archenteron is wide, the blastocœl apparently obliterated, and one of the two pole-cells is seen in the neighbourhood of the blastopore, although the relation of this cell to the two germinal layers could not be distinctly made out.

The fate of the blastopore is difficult to trace with certainty.

From a comparison, however, of numerous sections of L. Lep-toclini and L. Tethyæ at this and somewhat later stages, I have come to the conclusion that the blastopore closes in the position where the anus is subsequently formed, and that the œsophagus makes its appearance as a stomodæum in front or this region.

The mesoblast in Loxosoma originates in great part at any rate from the pair of pole-cells. In the later stages, as long as they are present, they are found in the immediate vicinity of the anus, each giving rise to a forwardly directed mesoblastic band. It is difficult by means of sections to deter-mine the period at which the anus originates, but even in the adult the anus can hardly be discovered except during the process of defæcation. I am unable to state positively how much of the alimentary canal is formed as a proctodæum, but I regard it as probable that the whole of the "rectum," as far as the constriction dividing the posterior limb of the alimentary canal into two parts (fig. 52), originates in this way; fig. 40, *an*, probably indicates the formation of the rectum as a proc-todæum. Whatever may be the real history of this part of the embryo, the intestinal end of the hypoblast seems to remain from the earliest stages in connection with the epi-blast, marking the spot where the blastopore either closes or persists as the anus. In front, however, occurs a large funnel-like epiblastic involution which forms the stomodæum, and early communicates with the archenteron. In the embryo fig. 38 it is probable that the stomodæum extends as far as the line *z*, the histological difference between the (supposed) epiblastic and hypoblastic cells being very conspicuous.

From a consideration of sections of L. Tethyæ it appears probable that some of the mesoblast may originate entirely independently of the pole-cells from any portion of the embryo, but in later stages of L. Leptoclini the main production of mesoblast is probably due to the pole-cells, as indicated by fig. 40. This is a section passing through one of the meso-blastic bands formed from a pole-cell. The commencement of the band is at the side of the anus, as shown by the position of

the vestibular invagination (*v.*), described later. The band consists of a series of branched cells, lying in the blastocœl ("primary body cavity" of Hatschek), in which are seen, in addition, three mesoblast cells, whose origin is uncertain. In the next stages the mesoblast consists of cells with anastomosing processes (figs. 41 and 42), scattered irregularly through the blastocœl, but I have never observed anything corresponding to a splitting of the mesoblast into splanchnic and somatic layers. Iu still later stages the mesoblast occurs mainly at the sides of the body, the more median regions being occupied by the alimentary canal, "dorsal organ," and "vestibular invaginations." The mesoblast cells, however, fill up almost all the interspaces between these structures, so that the mature lava is practically a solid mass of closely-packed tissues, this fact, of course, rendering it a difficult undertaking to distinguish the nature of all its constituent cells.

The disposition of the mesoblastic bands (which at first are situated near the ventral surface), and their relation to the pole-cells (which originate at the sides of the blastopore, and are subsequently found at the sides of the anus), seem to me to support the view that the blastopore and anus are identical in position.   .

The alimentary canal of the embryo is completed at an early stage, and subsequently undergoes no very striking changes until the larva becomes free. It consists in advanced embryos of the following regions, all distinctly marked off from one another: (i) the œsophagus (fig. 52, *œ.*) doubtless formed as a stomodæum; (ii) the stomach and intestine (*st.* and *int.*) passing quite gradually into one another. Dorsally and laterally the stomach is lined by low hypoblast cells, ventrally by cells which are high and columnar, nucleated peripherally, and containing yellow spherules; these cells, in fact, are the precise equivalent of the "liver-cells" of the adult; (iii) the rectum (*rec.*), separated from the intestine by a constriction, and probably formed as a proctodæum. The anus opens on a well-marked rectal cone projecting into the vestibule.

The alimentary tract of the larva thus resembles in every

particular that of the adult, in which can be similarly distinguished œsophagus, stomach (with "liver-cells" ventrally), intestine, and rectum, the various parts having exactly the same relations to the two limbs of the U-shaped gut in the adult and in the embryo alike. Both the mouth and the anus open within the circumference of the ciliated ring (*cr.*), with respect to which the larva is entoproctous, just as is the adult in relation to the lophophore.

Formation of the Sucker, Dorsal Organ, and Vestibule.—At the time when the alimentary canal is just completed a series of most important changes takes place in the constitution of the embryo, involving the appearance of the sucker (the homologue of the adult foot-gland), of the "dorsal organ," and of the vestibule. Fig. 37 is an almost median longitudinal section of an embryo at a a period when the sucker and the "dorsal organ" are still in a very early stage of development. The œsophagus (*œ.*) is cut medianly, the epiblastic invagination from which it has been formed extending as far as the mark *z*. The sucker (*s.*) is distinctly marked out as a group of epiblast cells much higher than their neighbours, and already indicated on the exterior by a slight depression of the surface of the embryo. The alterations by which the sucker passes into its permanent form are very unimportant, the most noticeable change consisting in a deepening of the involution, and an increase in the number and in the height of the cells composing the organ. The sucker, which is retractile, serves for the attachment of the embryo to the walls of the brood pouch, and this is one of the causes of the fact that it is functional at so early a stage. The foot-gland is no doubt the homologue of the larval sucker, with which it is much more similar in structure than could be supposed from previous descriptions of the adult organ. If my description of the latter is correct the main difference between the embryonic sucker and its adult representative consists in the elongation of the rounded depression, constituting the former, to the open elongated groove of the latter; in the adult, however, an additional complication has taken place in the separation of the ventral

portion to form the "gland," the "duct" of the organ
remaining throughout life fundamentally in its embryonic
condition. In fig. 37 the dorsal organ (*br*) is seen, contrary
to the statements of Hatschek on Pedicellina to consist of
a few columnar epiblast cells. The section in question is
only one of a considerable number, giving the same evidence
as to the earliest development of this structure. From its
position there cannot be any doubt that the epiblastic thicken-
ing is correctly identified as the dorsal organ, which neither
in its earliest appearance nor in its ultimate fate shows the
slightest evidence of its supposed function as a bud-producing
organ. At the earliest stage at which it is possible to identify
the dorsal organ with certainty, it invariably consists of a single
layer of cells forming part of the one-layered body wall, and
there can hence be no question as to its epiblastic nature.
One further point may fairly be urged against Hatschek's view
of the development of his "Entodermsäckchen." According
to Hatschek certain hypoblastic cells are budded off from the
anterior wall of the mesenteron; they enter into connection
with an epiblastic thickening, with which they form a budding
organ, the endoderm cells of the bud being derived from the
hypoblastic mass, which has taken origin from the walls of the
mesenteron. My own sections lead me to believe that the
stomodæum extends inwards a greater distance than was sup-
posed to be the case by Hatschek in Pedicellina (see figs.
37 and 38), and if my view is correct the dorsal organ makes
its appearance opposite a portion of the stomodæum. This
being the case, judging from Hatschek's description, the first
cells forming the dorsal organ, and occurring between the epi-
blast and the hypoblast, are derived from the epiblastic cells
of the stomodæum, and this hypothesis refuses to adapt itself
to Hatschek's own view on the nature of the budding process.
Finally, I must state that I have satisfied myself, by means of
sections, that even in the embryos of Pedicellina the dorsal
organ first appears as a thickening of the epiblast.

In order to prepare the way for the following description
of the further history of the development, I may at once state

my conviction that in the "dorsal organ" of the Entoprocta
we may recognise the supra-œsophageal ganglion or
brain of these animals.

The horizontal[1] section fig. 43 cuts the œsophagus (œ.)
and the intestine (int.); the epiblast is somewhat thick, as
this is the region of the ciliated ring. The space between the
alimentary canal and the epiblast has increased in size; on the
right side is a mass of mesoblast cells growing forward from
the region of the anus, the left wing of the mesoblast not being
seen, owing to a slight obliquity of the section. Between the
œsophagus and the intestine occurs a bilobed, apparently solid,
mass of cells which are derived from the epiblast, and repre-
sent an early stage in the development of the vestibule. Each
of the halves of the bilobed mass in reality corresponds to a
shallow depression of the ventral surface of the embryo in
front of the anus.

Fig. 41 represents a section of a somewhat later stage,
passing in an obliquely longitudinal direction and cutting one
side of the œsophagus (which is considerably elongated trans-
versely), and also a vestibular invagination. Behind the latter
occurs the commencement of one of the paired mesoblastic
bands, which is seen passing forwards and breaking up into an
irregular mass of branching mesoblast-cells. Anteriorly is
cut one of the two lateral halves of the brain, which has
already grown inwards as a pair of wings, one on each side of
the œsophagus. In a horizontal section of a considerably older
stage (fig. 44), each vestibular invagination is composed of
cells, whose nuclei are arranged in several layers. The section
shows anteriorly a portion of the ciliated ring, indicated by the
thickness of the epiblast and the elongation of the nuclei,[2]

[1] This term is used somewhat loosely in the description; in some "hori-
zontal" sections, for instance, the œsophagus and stomach are cut; in others
the œsophagus and intestine or rectum.

[2] The existence of the nuclei on the outer side of the cells of the ciliated
ring in the figure is due to the fact that a slight fold of the body wall has
been produced by the action of reagents, and has been involved by the
section; the cells of the ciliated ring are of course part of the external
epiblast.

characters retained in all subsequent stages ; throughout life it consists of a single row of cells bearing long cilia.

In a still later stage (fig. 45), the two vestibular cavities (*v.*) have fused in the middle line, and form a transversely extended groove separating from one another the process of the body bearing the rectum (*rec.*) and the epistome ; the mouth at this, as at other stages of the embryonic history, is a transversely elongated funnel, connected on each side with a groove which runs round the ventral surface of the body, just within the ciliary ring. This " oral groove " is represented in fig. 40, *og.*

Only the deeper portions of the vestibule are formed from the invaginations just described ; the peripheral parts originate from a growth ventralwards of the region of the body bearing the ciliated ring, which can be either extended to the exterior, as in swimming, or can be retracted into the interior of the vestibule, whose aperture is then constricted, as indicated in fig. 55, *va.*

We may now take up the further history of the dorsal organ or brain, which we left in the condition of a thickened area of the epiblast, composed merely of a single layer of cells. In fig. 38 the dorsal organ (*br.*) is seen to have become two cells thick. Fig. 46 represents a somewhat earlier stage, seen in horizontal section ; the epiblast, elsewhere very thin, has proliferated off a bilobed cellular mass, the wings of which pass as far as the sides of the œsophagus. The number of cells composing this structure, the brain, increases considerably, and before long it may easily be observed by means of horizontal sections (figs. 47 and 48) that the cells are arranged in a single layer round a lumen which opens medianly to the exterior. It is probable that the brain is at first formed by a solid ingrowth of cells, in which a cavity subsequently appears, although it is not impossible that a lumen may be present from the very commencement, and that owing to its small size it cannot easily be detected in sections. In either case, the brain is formed by a process of epiblastic invagination.

Hatschek (14) has already illustrated and described in

Pedicellina the stage corresponding to fig. 52 of Loxo-
soma; according to the statements of this observer, the
"Entodermsäckchen," originally a solid cell mass, becomes a
sac enclosing a lumen; the sac attaches itself to the epiblast,
and its lumen then opens (secondarily) to the exterior.   The
latter stage undoubtedly exists in Pedicellina, although, as
explained above, Hatschek's statement that the dorsal organ
contains hypoblastic elements is probably erroneous.   The
main difference between the brain of Pedicellina (for such I
consider to be the nature of the dorsal organ in this genus
also) and that of Loxosoma consists, at the present stage,
merely in its greater transverse elongation in the latter genus,
that of the former being represented by an oval sac whose long
axis is directed transversely.

Uljanin (5), it may be noted, states that in examining the
larvæ of Pedicellina, he at first erroneously supposed that
the dorsal organ and the sucker were nervous structures.   He
says (p. 437): "Sieht man eigenthümliche ganglienförmige
Organe, die mit einander durch Commissuren vereinigt sind.
Dieses Organ sieht einer Ganglienkette so ähnlich, dass ich es
auch anfangs für ein solches gedeutet habe.   Eine gründlichere
Untersuchung bewies aber dass eine solche Behauptung
nicht die richtige war .   .   .   .   im Innern dieser Organe
konnte ich, trotz aller meiner Bemühungen, nichts Ganglien-
zellen Aehliches finden."

In Loxosoma Leptoclini, before very long after the stage
represented in fig. 48, there appears on the deep surface of the
invaginated brain a layer of fibrous tissue (fig. 49 and fig. 52,
fbr.), which resembles the commissural part of the adult
ganglion in staining very slightly even after prolonged treat-
ment with borax carmine, or hæmatoxylin.   On each side of
the brain is now found a pigment spot or eye, whose mature
form is shown in the free larva, fig. 55, o.   The eyes make
their appearance at a time when the brain still possesses a
conspicuous lumen, which soon after their formation atrophies
by the mutual apposition to one another of the two cell layers
forming the walls of the sac; in the larva which is ready for a

free swimming existence, the brain has the appearance of
fig. 50 and fig. 51, two consecutive sections (horizontal) of the
same embryo. Fig. 51 shows the two eyes (*o*) which the
neighbouring section (fig. 50) has just missed, although it
passes through the fibrous region of the brain at its thickest
part (*fbr.*) On the outer side alone of the fibrous portion is
a mass of ganglion-cells, which in agreement with the mode of
their development are not arranged in a single layer. There
thus exists a well-marked distinction between the development
of the adult ganglion (suboesophageal) and that of the larval
brain. The latter is formed by a process of invagination, the
fibrous layer making its appearance entirely on the deep side
of the ganglion-cells, which are arranged in more than a single
layer; the former arises by the differentiation of a solid cell
mass from a thickened ectodermic region, the cells which com-
pose the ganglion separating from one another centrally, and
depositing a fibrous layer on their inner sides in such a
manner that the adult organ consists of a fibrous core sur-
rounded by a single layer of cells.

The finer histological details of the structure of the brain
cannot well be made out in paraffin sections, and in the fresh
condition it is even more difficult to investigate the minute
anatomy of the nervous system of the larva. From the·
examination of a very large number of sections of embryos of
various ages, I can assert the constancy of the relations to one
another of ganglion cells, fibrous layer and eyes, as described
above. It is especially important to notice that the fibrous
layer is invariably present, and can readily be found in any
series of sections passing through a mature embryo. The
existence of the eyes on the dorsal organ is a very strong
a priori argument for the occurrence of nervous tissue in this
region.

Fig. 54 is an obliquely horizontal section, passing through
œsophagus and intestine, brain, and vestibular invaginations.
On the left the section cuts the lumen of the latter, but on
the right only the cells limiting its blind end. Each of the
two invaginations consists of more than one layer of cells, and

anteriorly has given off a solid outgrowth which has met one end of the brain. This union of an outgrowth from each vestibular invagination with an end of the crescent-shaped brain is a constant occurrence; and it may be concluded that some of the deeper cells of the vestibular invaginations separate from the external epiblast to form a pair of suboesophageal ganglia, united with the brain (and with one another) by means of a circumoesophageal commissure. It must, however, be distinctly understood that the suboesophageal ganglion arises from the deeper cells of an epiblastic thickening, and not by a direct conversion of the vestibular invaginations into nervous tissue. The formation of the ganglion in the bud thus takes place in exactly the same manner as that of its homologue in the embryo. In stages more advanced than fig. 54, it is still possible to discover a commissure running from the brain on each side to a mass of tissue in the post-oral region, although at no period is it at all easy to observe the limits of the suboesophageal ganglion, which is closely packed with mesoblast cells, very difficult to distinguish in section from ganglion-cells.

The dorsal organ of Loxosoma, as is well known, is provided with a pair of ciliated sacs, one on each side, in the neighbourhood of the eyes, and opening to the exterior on its surface. These sacs are provided with active cilia, and extend inwards so as to lie in close contact with the external surface of the brain. As far as I am aware, the presence of these two sacs is the only foundation for the statement in Vol. i of Balfour's 'Comparative Embryology,' that the dorsal organ of Loxosoma is double, whereas that of Pedicellina is single. The brain of Loxosoma is in reality developed from an unpaired rudiment, and except for the fact that it extends further laterally than that of Pedicellina, there is no reason whatever for assuming its double nature. In Pedicellina the lumen of the brain atrophies, as already described by Hatschek; but before this process has taken place, there occurs, in the same position as in Loxosoma, a deposition of fibrous tissue, as I have been able to observe from sections. The

brain in Pedicellina, however, is not provided in any known species with eyes, this being in all probability a retrogression from a more archaic condition. An unpaired ciliated sac, described by Hatschek, is connected with the dorsal organ of Pedicellina.

These ciliated sacs of the Entoproctous larvæ are not to be confused with the primitive cavity of invagination of the brain, which loses its lumen during the conversion of its indifferent cells into nervous tissue; they are mere secondary epiblastic involutions, either developed for the aeration of the central nervous system or having the nature of olfactory organs, as suggested by Hatschek (17) for the similar structures in Polygordius.

The eyes of the larva of L. Leptoclini consist of crescentic reddish-brown masses of pigment (fig. 55, *o*), in whose concavity is imbedded a prominent transparent lens; the finer details of the structure were not made out.

Eyes are present in most larvæ of Loxosoma hitherto described, although they appear to be absent in L. pes (Schmidt, No. 11). The larva of this species differs to a considerable extent from that of L. Leptoclini. In Schmidt's figure (Taf. ii, fig. 25) there occurs nothing which can with certainty be identified as the dorsal organ, whilst the larva is provided with three pairs of bodies of an entirely problematical[1] nature. The larva of L. Tethyæ I find, in external features, to be almost exactly like that of L. pes figured by Schmidt; but at present I have not been able to elucidate the nature of the three pairs of problematical organs. Eyes are absent in the larva of L. Tethyæ, although I have succeeded in obtaining preparations which demonstrate the existence of a brain, especially unmistakeable in the stage represented in fig. 62 (*br.*). The dorsal organ of the larva of L. Tethyæ is, however, not nearly so highly developed as that of L. Leptoclini. By comparing the development of the latter with that

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[1] Schmidt, in his second paper (20), suggests that one of these pairs may constitute the "Knospenstöcke" or budding organs of the larva, identical with the structures described as such (probably erroneously) in the adult.

of certain other Trochospheres (as described below), we obtain evidence that the history of the brain in L. Leptoclini is really archaic in its general features, and there hence appears to be little reason for hesitation in the assumption that the larvæ of both L. Tethyæ and L. pes are degenerate forms, in which the sense organs (eyes) and the brain have suffered retrogressive modifications. A similar change, though to a less marked extent, seems to have occurred in the larva of Pedicellina, which in this respect thus shows itself to be less primitive than that of L. Leptoclini.

It has been suggested by Hatschek (14) that the dorsal organ becomes the first bud in Pedicellina, the first pair of buds in Loxosoma; in order to establish my view of its nature, it is necessary to examine the history of the metamorphosis, to ascertain if the larva makes use of its dorsal organ as a budding region. This I find definitely to be not the case in L. Leptoclini, and the hypothesis of the nervous nature of the dorsal organ remains in consequence unshaken when the budding processes of the larva are studied.

My method of obtaining free larvæ was to place a large number of L. Leptoclini growing on the Ascidian in a glass vessel, blackened in every part with the exception of a small window facing the light. On covering the top of the vessel with a piece of black paper, the larvæ, as they were hatched, made their way to the only part of the glass whence any light was proceeding, and in this position they could be easily detected and captured.

From various causes, and chiefly from the death and consequent putrefaction of the Leptoclinum within a day or two from its removal from the sea, the number of the larvæ which I succeeded in observing during the process of their metamorphosis was very small. In no single case did I observe any permanent fixation of the larva, but the method of the fixation, if this really takes place normally, is perhaps not of very great importance. If, as is probable, the normal resting-place of the larva is on the surface of the Leptoclinum which bears the adult individuals, the dying condition of the Ascidian in

captivity is sufficient to account for the refusal of the larvæ to attach themselves.

My observations on the metamorphosis of L. Leptoclini are exceedingly incomplete, but they have been sufficiently successful to demonstrate that the free larva may produce a pair of buds (fig. 55, *b*), although I am unable to say whether the budding takes place normally during a free life, or as a general rule only after fixation. In the most successful experiment, four larvæ were kept alive and apparently healthy for four days after the commencement of their free existence. They had produced buds considerably older than those represented in fig. 55, although, as in the adult, they were unequally developed on the two sides of the body. The older one of each larva was more elongated than those of the figure, and was somewhat pear shaped, being attached by its narrow end.

At its free end was an elongated slit, the opening into the vestibule, and these, the oldest larval buds observed, had exactly the appearance of those normally developed on the adults soon after the opening into the vestibule is a longitudinal slit on the anterior side. As I was not aware that I should fail in rearing larvæ a second time to the same age, I neglected to kill any of the four-day larvæ, which twenty-four hours later were found dead and disorganised. There could not, however, be the slightest doubt that the two structures described above were really correctly identified, and the larva of L. Leptoclini at any rate may be stated to produce a pair of buds in a position corresponding to that of the budding regions of the adult. The probability that this would be found to be the case had already been indicated by Hatschek, who made this suggestion on the hypothesis that the dorsal organ was the budding region. In my four-day larvæ, however, this structure showed not the slightest change from its condition at the commencement of the free existence; the position of the head was still continually varied for convenience of vision (this region of the body being exceedingly moveable), and the cilia in connection with the dorsal organ, like those of the epistome and ciliary ring, were working vigorously.

Fig. 55 represents a larva after a free existence of about twenty-four hours; the animal is seen from the ventral side, the ciliated ring being completely retracted into the vestibule, the narrowed opening of which is seen in the centre (*va*). By careful focussing could be made out the ciliary movements of the œsophagus and rectum, not indicated in the figure. The head or dorsal organ with its two eyes is very conspicuous and has in no way been altered in appearance by the production of the buds, which are seen as a pair of round projections at the sides of the dorsal organ, and at a level intermediate between it and the edge of the retracted vestibule. This position of the buds having been observed, it at once became obvious what was the nature of certain cells which had been repeatedly seen in the more advanced embryos within the brood pouch in exactly the position of the larval buds. These cells (fig. 57, *b*) form a group on each side, consisting of a small number of large rounded epiblastic cells lying at the sides of the brain, and between this organ and the ciliated ring. In larvæ one or two days free they are found to be more numerous and form a considerable projection composed in the main of enlarged epiblast cells. In fig. 59, a horizontal section of a larva which had been free two days, one of the buds (*b*) is cut on the left side. It consists externally of a layer of very high epiblast cells. On the inner side of these cells occurs a large rounded mass which seems to contain only a single nucleus. This large cell in all probability belongs to the epiblast, which I have observed, from an examination of other larval buds, to give rise to a mass of cells which projects inwards, and no doubt eventually forms the lophophore and vestibule, in the same manner as in the buds developed by the adult. The section has passed through the eye of the opposite side, together with a portion of the corresponding half of the brain, and it is easy to see, from an inspection of this and other series of sections of larvæ at the same or earlier stages, that the dorsal organ remains quite unaltered during the budding.

In fig. 56 are represented two groups of cells (*hst.*), apparently developed from the sides of the stomach. In larvæ

which have just become free occur two very conspicuous lateral masses of large cells (figs. 50 and 51, *hst.*) lying between the epiblast and the stomach, and corresponding to those which seem to be originating in fig. 56. In larvæ which have been free two days, the lumen of the stomach is almost completely atrophied (fig. 58, *st.*), although the œsophagus and intestine are much less altered. The figure represents the œsophagus at the point where it passes into the stomach, whose "liver-cells" are still recognisable (*l.*). At the sides of the stomach are two masses of cells, probably corresponding to those developing in fig. 56; that of the left side is seen to pass to the base of the bud, whose outer portion is just involved by the section. In the other sections passing through the same larva, no stomach lumen could be discovered, but only a mass of cells (represented in fig. 59), which fill up the whole of the central portions of the larva, and which pass to the bases of the bud. These appearances, which are not confined to a single larva, have led me to form the following hypothesis of the budding processes, in default of a complete series of actual observations.

One of the earliest formed parts of the bud is the epiblastic thickening, which occurs long before the embryo is ready to become free, and during embryonic life consists of a small number of large granular cells. Almost at the same time are proliferated off from the sides of the stomach a pair of lateral wings of hypoblast cells, destined to take part in the budding. After the commencement of the free life these cells increase in number by subdivision, new ones being at the same time formed from the walls of the stomach, which atrophies coincidently with their formation. The stomach is thus completely lost, and is replaced by a large mass of cells filling up the centre of the larva. The epiblastic thickening meanwhile has grown inwards, and the bud forms a projecting rounded lobe of the body of the larva. The epiblastic portion forms the lophophore and vestibule, the latter originating as a longitudinal slit, just as in the buds formed on the adult. The central mass of hypoblast cells is employed mainly as a food material during the development of the bud, but some of these cells

apply themselves to the deep end of the lophophore, and give rise to the stomach of the adult. I have one preparation of a larval bud, which seems to show this application of a hypoblast cell to the end of the lophophore.

The origin of the various organs in the buds developed on the adult has been a subject of much controversy. The bud commences as an ectodermic thickening, growing inwards to form the lophophore. It is very difficult to determine satisfactorily whether the endoderm develops from cells proliferated from the stomach or from the ectodermic thickening which occurs at the apex of the bud. According to most observers the latter is the case. Although at present I have been unable to satisfy myself on this point, it has seemed to me, on the whole, probable that in the adult the stomach does give rise to cells, which apply themselves to the end of the lophophore, and are destined to form the stomach of the bud. I have been unable to convince myself of the accuracy of Haddon's account (37), according to which the stomach at its earliest appearance is quite distinct from the lophophore. My own sections lead me to believe that the stomach is connected with the lophophore, even in its youngest condition, and that the only method by which it can have an endodermic origin is by the proliferation of cells from the stomach, and by the application of these cells to the ectodermic part of the bud. The ectoderm and endoderm on this hypothesis become indistinguishable, so that at a later stage the stomach appears to be developed from part of the ectodermic thickening forming the lophophore.

The most noteworthy features of the history of the larva to which it is necessary to call attention are (i) the fact that the dorsal organ takes no part in the budding, and (ii) the atrophy of the stomach after the commencement of the free larval existence. The latter fact probably indicates that the larva never becomes adult, but that it dies after the production of its buds. Although it is organised on a plan fundamentally similar to that of the adult there is still a very well-marked distinction between the anatomy of these two forms, and in the four days during which I succeeded in keeping some of my

larvæ alive there was not the slighest indication of the loss of any of the larval characters or of the approximation, in a single feature, to the adult condition.   The (probable) fact that the larva does not become adult throws some light on the absence of the brain in the stalked Loxosoma, since we may assume that this organ has been inherited by the larva from a brain-possessing ancestor, but has been transmitted to none of the individuals produced by budding, and whose development cannot be regarded to any great extent as a recapitulation of the phylogeny of the Entoprocta.

In experimenting with the larvæ of L. Tethyæ and Pedi-cellina I have completely failed to obtain any indications of the processes of the metamorphosis.   This is especially striking, since on one occasion larvæ of the former were kept alive for as much as eight days after they were hatched ; at the end of this time they were still free-swimming, and, externally at any rate, showed no obvious indications of buds.

Part 4.—On the Affinities of the Polyzoa.

The embryology of Dentalium has recently been inves-tigated in considerable detail by Kowalevsky (36) ; the agree-ment between this description and my own observations on Loxosoma is very striking, especially with reference to the nervous system.   The alimentary canal of Kowalevsky's larva possesses the characteristic Trochosphere bend, whilst the mesoblast develops from a pair of pole-cells in the neighbour-hood of the blastopore.   The shell-gland appears dorsally at an early period (whilst the embryo is still a gastrula), and has at a certain stage the form of an involuted sac, whose similarity in Molluscs in general to the foot-gland of Loxosoma has already been pointed out by Lankester (8).   In the anterior region of the embryo, in the middle of the velar area, originates a pair of separate epiblastic invaginations, the "sincipital tubes" of Kowalevsky.   These tubes grow backwards into the præoral lobe for a considerable distance, ending blindly one on

each side of the œsophagus.   Even at a late stage, each pos-
sesses its own opening to the exterior, although early in their
development the two tubes become connected by a median
bridge of subepithelial epiblast cells.   Eventually their lumina
disappear, and they are either partially or completely converted
into the nervous tissue of the brain, Kowalevsky inclining to
the latter hypothesis.   The manner in which the median com-
missure connecting the two halves of the ganglion is developed
has not been satisfactorily followed.   In the young Denta-
lium the brain is a transversely elongated structure in front
of the œsophagus, consisting of a central fibrous core sur-
rounded by ganglion-cells.   The similarity between this
ganglion and the brain of the larva of L. Leptoclini or the
ganglion of the adult Loxosoma, is so striking that I have
reproduced Kowalevsky's fig. 92 in my own fig. 23 for com-
parison with figs. 50 and 51 of the brain of the larva of
L. Leptoclini, and with fig. 16 of the ganglion (sub-
œsophageal) of the adult L. Tethyæ.   When it is remem-
bered that neither of these organs in Loxosoma has hitherto
received the interpretation suggested in this paper, it will
probably be admitted that the histological characters of the
structure which is undoubtedly the brain of Dentalium,
confirm very strongly the view that the above-mentioned
organs of Loxosoma are similarly nervous in character.

The pedal ganglia of Dentalium develop as paired thicken-
ings of the epiblast at the sides of the foot, not far behind the
œsophagus.   They originate quite independently of the cephalic
ganglion, with which a secondary connection is established ;
and this, as Kowalevsky points out, is in all probability cha-
racteristic of the development of the Mollusca in general.[1]

In both Loxosoma and Dentalium the brain originates
by a process of invagination, the subœsophageal ganglia (pedal)
as paired thickenings of the epiblast, and not, like the brain,
as invaginations, whilst the connection between the two por-

[1] In the same way, in Lumbricus trapezoides (Kleinenberg), the
brain and ventral cord originate independently and become secondarily con-
nected (Balfour, ' Comp. Emb.,' vol. ii, p. 336).

tions of the nervous system is secondarily established. The existence of two cephalic invaginations in Dentalium in no way impairs the comparison which has just been instituted between it and Loxosoma, where a single invagination occurs. Nothing could show this more clearly than the manner in which the brain develops in some Pteropods (Hyaleacea), described by Fol; this may be explained by the quotation of a few lines from Balfour ('Comp. Emb.,' i, p. 227) : " A disc-like area appears in the centre of the velum, which soon becomes nearly divided into two halves. From each of these there is formed by invagination a small sack. The axes of invagination of the two sacks meet at an angle on the surface. The cavities of the sacks become obliterated ; the sacks themselves become detached from the surface, fuse in the middle line, and come to lie astride of the œsophagus." This mode of the development of the brain forms a most instructive transition from Dentalium to Loxosoma. In Dentalium the two halves of the brain are separate invaginations, extending inwards for a considerable distance parallel to the long axis of the embryo. In the Pteropods just described, the axes of invagination are inclined to one another on the surface, their apertures being situated close together ; and by supposing the invagination in the latter case to encroach on the middle line, the two apertures would fuse, and we should have precisely the same arrangement as that of Loxosoma represented in fig. 48.

It seems to me that if we once admit the nervous nature of the parts which I have described as such in Loxosoma, the identification of the surfaces in the Entoprocta is no longer a matter of doubt. The dorsal organ, if a ganglion at all, must be supra-œsophageal, from its position, from the mode of its development, and from the fact that it is provided with paired eyes (doubtless true cephalic eyes inherited from an ancestor common to the Trochosphere group, and not accessory eyes like those developed on other parts of the body in many Ectoproctan larvæ).

Caldwell (34) has recently suggested a view of the body plan

of the Polyzoa, based on an investigation of the metamor-
phosis of Actinotrocha; he states that "the dorsal surface
in Polyzoa is indicated as in Phoronis by the line between
mouth and anus," and gives the following explanation of his
views on the larvæ : " The larvæ of Brachiopoda and Polyzoa I
regard as modified from the Trochosphære by the earlier
attainment of the relation of the ventral surface which in
Phoronis is only accomplished during the metamorphosis."
In Actinotrocha, the larva of Phoronis, the surfaces
correspond to those of ordinary Trochospheres, but their
relations become much distorted by the formation (by invagi-
nation) of a large ventral sac between mouth and anus,
subsequently everted as the " foot," into which the alimentary
canal passes.   The Trochosphere bend of the alimentary
tract is thereby exactly reversed, the " foot " becoming the
body in which the gut forms a U-shaped tube with a dorsal
flexure.   Caldwell's suggestion is that this dorsal flexure of
the alimentary tract has become already acquired during the
larval condition of Polyzoa and Brachiopoda.   In the
developmental history of Loxosoma, however, there is not
the slightest trace of the existence of a flexure of the alimen-
tary canal towards the dorsal surface.   We have already
compared the larva of Loxosoma with that of Dentalium,
an admitted Trochosphere; we have found that in both
forms the mesoblast develops as two lateral masses near the
surface containing the mouth and the anus.   Further, the
comparison already instituted between the dorsal organ of
Loxosoma and the brain of Dentalium, between the sub-
œsophageal ganglion of the former and the pedal ganglia of
the latter, seems to leave no doubt that the dorsal organ of
Loxosoma represents its præoral lobe, and as such a part of
the dorsal and not of the ventral surface.   As an additional
indication of the surfaces, I must insist on the position of the
apertures of the nephridia between mouth and anus, and in
front of the (supposed) subœsophageal ganglion.   On Cald-
well's hypothesis, we must regard the adult ganglion as the
brain, and the curious result follows that the head-kidneys

open dorsally on the præoral lobe, between the brain and the mouth !

Lankester (45 and 49) has adopted Caldwell's explanation of the surfaces of the Polyzoa as the most probable which has been suggested ; the buccal shield of Rhabdopleura, and therefore, one may conclude, the large epistome of Loxosoma, being considered possibly a representative of the mantle area.

Lankester's earlier opinion (7 and 8) on the relations of the surfaces of the Polyzoa is well known, the epistome being homologised with the foot, and the foot-gland with the shell-gland of Mollusca. This opinion has of course been re-nounced in accepting Caldwell's view of the Polyzoa.

Balfour (28) has urged two difficulties against the view of the homology of the foot-gland (undoubtedly the same organ in both the adult and the larva of Loxosoma) with the Molluscan shell-gland. These difficulties are—(i) the ciliation of the foot-gland of the larva; (ii) its relation to the velum. The sucker of the larva of the Entoprocta is not, however, invariably ciliated ; it is indeed surrounded by a circlet of stiff hairs, probably tactile like the sense hairs of the adult Loxosoma or of the larval Pedicellina; but it seems quite natural to suppose that special tactile organs would be advantageously localised in the neighbourhood of any apparatus commonly used for the temporary attachment of its possessor o foreign objects. The presence of these sensory hairs, quite similar to those occurring in other regions of the body, need in itself form no valid objection to Lankester's (former) view. The shell-gland of Mollusca is not used for attachment, and does not require a development of these tactile hairs.

The objection which refers to the position of the velum appears to me of a more serious nature. The ciliated ring of Loxosoma has generally been regarded as homologous with the velum of Trochospheres, and on this supposition the ciliated furrow running along the inner border of the ring (in the larva of Loxosoma) and produced by means of the oral groove into the œsophagus, corresponds with the similar ciliated

area which Hatschek (17) finds to be invariably present behind the velum in Trochospheres.

The shell-gland of Mollusca is commonly situated more posteriorly than the sucker of Polyzoa; it is not impossible that if it were once used for purposes of temporary attachment, it would be most conveniently situated in the centre of the aboral surface, in order to allow the cilia connected with the mouth to assume the position most favorable for nutritive purposes. The shell-gland might thus travel somewhat forward as a result of its new function, the cilia concerned in nutrition and locomotion alike becoming confined simultaneously to the ventral surface.

Hatschek (17) has pointed out that in Polygordius the ciliary apparatus (velum and oral cilia) does not originate as a closed ring; it appears first on the ventral side, and subsequently completes itself dorsally. This mode of development is also characteristic of some Gastropod Trochospheres, as shown by Bobretsky (48). Thus in the phylogenetic history of the Entoprocta, it is possible that when the sucker was situated more posteriorly, the velar ring was at first completed on its anterior side; that as the sucker travelled forwards, it reached a point where it interrupted the velum dorsally; and that finally, when it had arrived at a subcentral position, the posterior union of the two halves of the velum took place behind it, thereby increasing the efficiency of the nutritive cilia, when the animal was fixed. It is worth noting that in Dentalium, Kowalevsky (*loc. cit.*) describes the shell-gland as originating during the gastrula stage, whilst in Loxosoma the sucker similarly develops exceedingly early, and is functional whilst the brain, for instance, is still quite rudimentary. Although this early appearance of the sucker in Loxosoma may be merely due to the fact that it is an important embryonic organ, the period of its development, as well as its striking similarity to the young shell-gland of Mollusca, renders possible the view of its homology with the latter. The constancy of the occurrence of the shell-gland in larval Mollusca and of the sucker in embryos of Polyzoa (Ento-

procta and Ectoprocta alike) indicates the probable phylo-
genetic importance of these structures in the two groups.

Both the Molluscan foot and the Polyzoan epistome are
ventral outgrowths of the body situated between mouth and
anus, and with each of these organs is connected a pair of
subœsophageal (pedal) ganglia. The foot in Gastropod
Trochospheres (Bobretzky, No. 48) bears a striking resem-
blance to the epistome of Loxosoma ; it forms a conspicuous
prominence immediately behind the mouth (see fig. 106, vol. i,
of Balfour's ' Comp. Embryology '). It is separated from a
region bearing the anus by a deep transverse groove, which on
the supposition that the epistome represents the foot of Mol-
lusca would correspond with the vestibular invaginations of
the larval Loxosoma or with the median depression behind
the epistome in the adult.

Professor Lankester has kindly furnished me with a proof of
his article "Polyzoa," shortly to appear in the ' Encyclopædia
Britannica.' In considering the phylogeny of the class, Lan-
kester has adopted the view that the Entoprocta do not in
reality exhibit archaic characters, but that they have been
derived from forms like Phoronis with a hippocrepian lopho-
phore, a large cœlom, and a dorsal flexure of the gut. There
is, however, really no evidence that the circular Entoproctous
lophophore is a special modification of the horse-shoe-shaped,
and it appears to me more natural to consider it as replacing
the larval ciliated band, which probably represents the similar
structure in Trochospheres. My conclusions with regard to
the surfaces of the larva are diametrically opposed to those of
Lankester, and I must again refer to the characters of the
nervous and excretory systems as indicating the relations of
the Entoprocta to other animals.

On the assumption that Loxosoma is not an archaic form,
it is necessary to show that the complicated ontogeny of the
Ectoprocta has been here replaced by a direct development,
whereby the enigmatical larval organs of these forms have
been lost.

It appears to me, however, that the development of the

Entoprocta is not in reality direct. The larva, it is true, possesses many organs which have their homologues in the adult; but I have already explained my reasons for believing that it does not itself become mature, and that the permanent individuals are produced indirectly by budding. Lankester's theory affords no explanation of the occurrence of the dorsal organ, a structure which is entirely confined to the larva.

Hatschek (17) has exemplified by means of two diagrams (p. 106) the homologies between a Pedicellina larva and that of an Annelid; he points out that the only difficulty in the comparison is the fact that the "Scheitelplatte" of the former has never been identified, although he suggests that this structure may be represented by the sucker. His diagram is, as I believe, perfectly correct in most of its essential features :— in the identification of the surfaces, in the relations of the parts of the alimentary canal, in the position of the ciliated ring, of the head-kidney, of the suboesophageal ganglion, and of the pole-cells of the mesoblast. The only characters in the diagram to which I take exception are (i) the identification of the sucker instead of the dorsal organ, as the "Scheitelplatte," or brain; and (ii) the opening of the nephridium into the body cavity by a ciliated funnel (which Hatschek supposed to be also the case in the Polygordius larva).

Leaving entirely out of consideration the ciliated ring, the sucker or foot-gland, and the epistome, all of them structures whose homologies are uncertain, the Entoprocta, adult and larval, remain essentially modified Trochospheres. The existence of paired nephridia, corresponding to the head-kidney of Annelid larvæ, their inner ends situated in the primary body cavity, and their external apertures in front of the suboesophageal ganglion, seems to me of especial importance in the consideration of this view. The absence of anything corresponding to a ciliated funnel, opening into a secondary body cavity, is again of importance. The identity of the gut-flexure of the Entoprocta with that of Trochospheres has not been disproved by Caldwell, who has failed to bring forward a single argument against the ordinarily received view

of the surfaces of the Polyzoa, but has based his conclusion entirely on a study of Phoronis. When we add to these facts the position of the nerve-ganglia and of the pole-cells of the mesoblast, I consider that it is impossible to resist the conclusion that Phoronis in no way gives an explanation of the anatomy of the Polyzoa.

Before passing to a consideration of the larvæ of the Ectoprocta it will be necessary to examine the statements of Uljanin (5), van Beneden (1), and Barrois (13, 29, and 33), with regard to the metamorphosis of Pedicellina. Uljanin's statements have already been shown by Barrois (13) to be based on the supposition that adult calyces fallen from their stalks were larvæ which were commencing to undergo their metamorphosis. Van Beneden's account and those of Barrois differ in fundamental particulars, and although it is conceivable that there may really exist a great difference in the post-larval stages of various species, it is very possible that van Beneden's account is incorrect. According to this observer we have in Pedicellina a metamorphosis of the simplest kind; the larva fixes by its sucker, this extremity growing out into a stalk, and a circlet of tentacles appears on the inner side of the ciliated ring, which is subsequently atrophied. If this account is correct (and it is not impossible) the first individual of the colony is the fixed larva.

Barrois's account (33) of the post-larval changes (in the Entoprocta in general) is the following :—(i) The larva fixes by its oral face; (ii) the vestibule sinks to the interior, and becomes divided into several portions : (a) an "inferior" portion corresponding to the outer part of the vestibule, that carrying the ciliated ring; this gives rise to the foot-gland, permanent in Loxosoma, temporary in Pedicellina; (b) a "superior" portion, the deeper part of the vestibule; and (c) a middle portion, forming a mass of globules filling the stalk. The portion described as b remains attached to the alimentary tract, which twists round (the details of this process are not given), so that the concavity of its flexure is ultimately directed towards the free surface of the fixed larva instead of towards

the attached oral face, as was previously the case. The ecto-
derm of the free surface (aboral of larva) now becomes
thickened ("labial thickening"), and subsequently invaginated
to meet the part of the vestibule connected with the reversed
gut. Into this portion of the vestibule the labial "invagina-
tion" opens, and forms the outer part of the permanent ves-
tibule. The oral face of the larva thus becomes the aboral side
of the adult, and *vice versâ*, and the foot-gland of the adult
Loxosoma is not homologous with the sucker of its larva.
The sucker and the dorsal organ are both derived from the
two primary layers of the embryo, but are simply larval sense
organs of no morphological importance; they disappear after
the metamorphosis.

By comparing these somewhat remarkable statements with
figs. 13, 14, and 15 of Pl. II of Barrois's earlier memoir (13),
it has appeared to me possible to suggest an explanation of the
"metamorphosis," at least as probable as that of Barrois him-
self. I would suggest (1) that the post-larval changes consist
in a process of budding; (2) that the surfaces of the adult are
in every way homologous with the similar surfaces of the larva,
so that the foot-gland of the former (Loxosoma) is the
actual homologue of the sucker of the latter; (3) that the
"labial thickening" of the fixed larva is the epiblastic thicken-
ing which probably gives rise to the whole of the vestibular
cavity of the first individual formed by budding; (4) that the
changes of the larval vestibule and alimentary tract described
by Barrois consist in their atrophy, as in Loxosoma, de-
scribed above, after the fixation, the hypoblastic cells of the
alimentary tract probably giving rise to the stomach and
other endodermic (?) tissues of the bud, as would seem to be
the case according to Barrois's own description; (5) that the
sucker and the dorsal organ, like the other purely larval struc-
tures, atrophy during the budding.

If the post-larval changes are not to be regarded as a meta-
morphosis, but as consisting in the production of a new indi-
vidual, it is conceivable that the larva might just as well fix
itself by its oral as by its aboral face, whereas, if it becomes

adult, it is almost inconceivable that it should not attach itself
by the end which bears the sucker. On the theory that the
process is a mere metamorphosis, the fact that the larva should
fix by its oral face, and that it should be thereby obliged to
develope a new vestibule, mouth, and anus at its primitively
aboral end, is an occurrence of the most extraordinary nature.
The metamorphosis of a more or less bilaterally a symmetrical
Echinoderm larva to the radiately-arranged adult form is not
a parallel case. The sexually mature Pedicellina has pre-
cisely the same general arrangement of its organs as its larva,
whose most direct way to become adult would consist in the
development of a stalk and budding stolon at the aboral end,
with the possible atrophy of the dorsal organ. The whole life
cycle of the Entoprocta appears to me to consist in a division
of labour, leading to an alternation of generations; the indi-
viduals produced from the egg having given up the sexual
function, but having retained many of the ancestral features
which favour a free pelagic existence; the individuals produced
by budding having, on the contrary, retained the generative
function, but having acquired a sessile habit. The organisa-
tion remains, however, fundamentally the same in larvæ and
adults alike, and both kinds of individuals are capable of the
production of buds, one of the most characteristic features of
the Polyzoa. Barrois' account probably shows that the larval
Pedicellina produces a single bud instead of two, as in
Loxosoma. Pl. II, fig. 14 (loc. cit.), represents a stage some
time after the fixation. In this individual can be distinguished
a disc of attachment, and connected with it a cylindrical body,
at whose apex is a young vestibule with its tentacles. The
proximal portion probably represents part of the larva, which
is fixed by its oral face; the larval organs have degenerated,
but the bud is already well developed. The nature of this
process is here less obvious than in Loxosoma owing to the
absence of any constriction separating the bud from the larva.

Of all the Ectoprocta, with the details of whose development
we are acquainted, Cyphonautes, the larva of Membrani-
pora, appears most easily comparable to that of the Ento-

procta.   The most important feature of Cyphonautes is the
fact that the alimentary canal is well developed in the larva ;
broadly speaking, it has the same arrangement as that of the
Entoprocta, and is obviously functional during larval life,
as can be concluded from the presence of food particles in the
stomach, as indicated by Repiachoff (27).   In the paper just
referred to, Repiachoff has given figures of Cyphonautes,
which I have been able to understand, partially at any rate,
by means of a short description in the ' Zool. Anzeiger ' (22)
(the longer paper is in Russian).   It is pointed out in this
paper that the "bud" described by Hatschek (14) in
Cyphonautes consists of two parts :[1] (i) an invagination,
composed of columnar cells opening into the anterior side of the
vestibule at its extreme ventral edge (fig. 24, *zc*) ; this portion
is surrounded by a ring of cilia ; (ii) of a mass of cells, cor-
responding to the " Entodermknospe " described by Hatschek
in Pedicellina, situated on the dorsal side of the invaginated
part, and apparently (from another figure of Repiachoff's)
giving rise to the first polypide of the colony.   It appears to
me not impossible, from this description, that the first part,
the invaginated sac, corresponds to the epiblastic invagination
which forms the dorsal organ of Loxosoma or Pedicellina.
By reflecting the anterior portion of the body of an Ento-
proctan larva into the vestibule, as Hatschek has suggested
(although with a different identification of the dorsal organ),
the aperture of the invagination might come to lie within the
area of the latter, and the involuted sac (*zc,* fig. 24) may thus
possibly represent the dorsal organ, which, instead of develop-
ing to a functional ganglion, remains in its embryonic con-
dition as a mere rudiment.   The mass of cells between the sac
zc in Cyphonautes and the sucker on the dorsal side of the
larva, appears from Repiachoff's fig. 2 to be concerned in the
larval budding ; and it is important to notice that in the figure
it is in intimate connection with the epiblast of the anterior
ventral portion of the vestibule, but not with that forming the

[1] See fig. 24, in which are reproduced the essential details of Repiachoff's
Taf. i, fig. 1.

outer body wall of the larva, lending support to the view that the anterior portion of the body is here reflected into the vestibule. If, therefore, the invaginated sac corresponds to the brain of Loxosoma, the budding regions in Cyphonautes and Loxosoma will also correspond (by imagining an eversion of part of the vestibule in the former). In producing a single bud, however, Cyphonautes probably resembles the larval Pedicellina.

From Repiachoff's account (18) of the development of Tendra zostericola, one of the Cheilostomata, it may be seen that the early processes are not unlike those of Loxosoma. At the morula stage there is a narrow blastocœl, the cells of the dorsal side being smaller than those of the ventral side. The latter are now invaginated, forming an archenteron, which loses its connection with the exterior. The account of the development may from this point be carried on from No. 21 in the list of references, aided by figures in the Russian paper already referred to. The epiblast thickens ventrally, and is invaginated as the "Saugnapf" (shown in a later stage (v) in fig. 22, a reproduction of Repiachoff's Pl. ii, fig. 5). In front of this "sucker," which must not be confused with the larval foot-gland, appears a stomodæum which meets the archenteron, and the latter gives off anteriorly at the point of union with the œsophagus, an outgrowth (hypoblastic) which segments off as a mass of cells compared to Hatschek's "Entodermknospe." Just in front of the œsophagus is an invagination of the epiblast (fig. 22, x), probably (as I conclude from the figure) corresponding to the invaginated sac of Cyphonautes; whilst dorsally occurs an epiblastic thickening (y) representing the foot-gland of the larval Loxosoma.

If this account of Repiachoff's is correct (it is doubted by Barrois, No. 23), it seems to me that it forms an important clue to the structure of Cyphonautes and of other Ectoproctan larvæ. (I am unable to say what view Repiachoff takes of the subject in his Russian paper.) The explanation which I would suggest is the following. The curvature of the alimentary

canal is the same as in Loxosoma, the stomodæum being homologous in the two cases; Repiachoff's "sucker" (*v*, fig. 22) represents the vestibular invaginations of Loxosoma, as has been pointed out by Barrois (33). The identity in general relations between these structures in Loxosoma after their median fusion (fig. 45, *v*) and the "sucker" of Repiachoff's Tendra (fig. 22, *v*) is in fact very apparent.

The hypoblast cells budded off from the front of the archenteron represent, on my view, not Hatschek's dorsal organ, but the cells which I believe to be proliferated from the larval stomach (long after the development of the dorsal organ) and to enter into the composition of the bud. The cephalic ganglion of Loxosoma would in this case be represented by the epiblastic invagination *x* of fig. 22, the relations of this to the budding organ being possibly different from that of the same structure in Cyphonautes, although this does not absolutely follow from the figure.

Barrois (23), in describing the development of Lepralia unicornis (Cheilostomata), confirms Repiachoff's account of the invagination of the archenteron, and further calls attention to two lateral mesoblastic bands at the sides of the blastopore. In a subsequent paper on the metamorphosis of Ectoprocta (33), Barrois has suggested, as mentioned above, the homology of his "internal sac," Repiachoff's "sucker," previously described by Barrois himself (13) as "stomach," with the vestibular invaginations of Entoprocta, although his identification of other parts appears to me unsatisfactory. During the metamorphosis, the larva is attached by the evagination of this "internal sac," and a complicated process takes place, resulting in the formation of the first polypide of the colony. From the organ described in Balfour's 'Text-Book' as the "ciliated disc," and which one might suppose to correspond to the foot-gland of Loxosoma (this view is controverted by Barrois) develops an invagination which forms the polypide. The eversion of the "internal sac" during fixation does not seem to me to preclude the possibility of its homology with the vestibular invaginations of Loxosoma;

the bud, as one may assume the young polypide to be (Barrois
takes the view that it is not a budding process), in this case
occupies a position which is by no means unlike that of the
young bud of Cyphonautes and of those of Loxosoma,
that is to say, it occurs on the dorsal side of the ciliated ring.
It is possible that in many Ectoprocta the dorsal organ, and
even the foot-gland, may be completely lost at all stages of
development, as if Barrois is correct in stating that the poly-
pide (possibly only its tentacle sheath and the organs con-
nected with it?) develops from the "ciliated disc," it is
obvious that the latter cannot entirely correspond with the
sucker of Loxosoma, but must represent in addition part of
the region situated more anteriorly in the latter.

It seems to me that a consequence of the facts known with
regard to the development of Membranipora and Tendra
must be an inquiry as to the validity of the assumption that
the Cheilostomata form the most modified of the groups of
the Marine Polyzoa, a conclusion, I believe, based mainly on
the characters of the aperture of the zoœcium or "cell."

It is now necessary to consider the possible relationships of
the Polyzoa with Phoronis and the Brachiopoda.

Caldwell has stated that "the identity of the Phoronis larva
up to the formation of the nephridia, and before the outgrowth
of the anal region, with the Trochosphære type of Hatschek
is complete," and as far as the larval characters go, I am ready
to admit that there is probably a real affinity between Phoronis
and the Polyzoa. From the commencement of the meta-
morphosis of Actinotrocha, however, it seems to me that
every step taken towards the attainment of the adult condition
is a step away from the Polyzoa.

The permanent nephridium of Loxosoma is the head-kidney
which forms a provisional excretory organ in Actinotrocha
and other Trochospheres. Thus the adult Loxosoma, in
the absence of the "secondary body cavity" and of the
ciliated funnel of the nephridium, and in the retention of the
"primary body cavity" of the larva, has remained at a grade
which is passed by Phoronis long before the adult condition

is reached. The larval Phoronis resembles Loxosoma in the possession of a ventral flexure of the alimentary canal, the dorsal gut-flexure of the adult being entirely unrepresented in the Entoprocta. Thus the epistome of Polyzoa is ventral, and the ganglion developed in its neighbourhood is to be homologised with the pedal ganglion of Molluscs rather than with the brain of Phoronis.

The vascular system of Phoronis is entirely unrepresented in the Entoprocta, whilst the asymmetrical generative organs of the former differ markedly from the simple paired gonads of the Entoprocta. The four divisions of the alimentary canal, characteristic, according to Caldwell, of Phoronis and Brachiopoda alike, are not represented in the Polyzoa, whilst the nervous system, which in Phoronis consists of a plexus of fibres and cells lying outside the basement membrane of the epidermis, and concentrated in certain definite regions, is in striking contrast with that of Loxosoma. It seems to me, in fact, that a comparison between the adult Phoronis and Loxosoma is exceedingly difficult, whereas in many of the Trochosphere characters of the larvæ it is easy to point to parallels in the two genera.

It is not altogether impossible that the invagination occurring on the ventral side of Actinotrocha, and subsequently evaginated to form the foot or body of the adult Phoronis, may be represented by the vestibular invaginations of Loxosoma. In the Ectoprocta, according to the researches of Barrois, fixation universally takes place by the evagination of the "internal sac" (the homologue of the vestibular invaginations). If instead of losing their stomach at an early period of development the Ectoproctan larvæ retained this organ, and became adult, it would not be impossible for the gut to pass into the evaginated "internal sac," and thus to reverse its former curvature, as in the case of Phoronis. I believe that the question of the relationship of the Polyzoa to the Brachiopoda can only be satisfactorily resolved by a renewed study of the embryology of the latter. Caldwell has laid special stress on the resemblances between Brachiopoda

and Phoronis, and if any affinity between the two does really exist, it follows that the Brachiopoda are not totally unrelated to the Polyzoa, although the question still remains whether the latter approach most closely the Trochospheres of Chætopoda, Mollusca, Phoronis, or Brachiopoda. Judging by the descriptions we at present possess, the Brachiopod larva is much further removed from the Trochospheral type than those of any of the other groups we have considered. If the points in which it differs from the typical Trochosphere are such as imply a real dissimilarity, and are not merely secondary larval characters, then it follows that the similarities between the Brachiopoda and the Polyzoa have no phylogenetic significance. The character of the lophophore has always formed one of the main reasons for associating together the Polyzoa and the Brachiopoda, and its arrangement in the form of a horse-shoe in Argiope (for instance) and the Phylactolæmata has usually been considered a proof of the affinity of the two groups. If, however, the Phylactolæmata are not so primitive as the Entoprocta, the hippocrepian character of their lophophore cannot be regarded as an archaic feature, but has been secondarily acquired. It seems to me, however, that a comparison between the developmental history of the Polyzoa and that of the Brachiopoda serves to show a considerable difference in the lophophores of the two groups. In Argiope (*vide* Oehlert and Deniker's abstract of Kowalevsky's Russian paper, No. 40), after the mantle lobes have turned forward and the tentacles have begun to make their appearance, the young Brachiopod has a striking superficial resemblance to an adult Loxosoma. But by comparing the latter with its larva, we find that its lophophore replaces the larval ciliated ring, and that the head is outside the circlet of tentacles. In Argiope, however, the mantle lobes are developed entirely behind the cephalic segment which bears the eyes, and probably represents the head, so that when they bend forwards after fixation, the part of the body bearing the eyes remains within the mantle cavity, a condition entirely different from that of

Loxosoma. The tentacles of Argiope are formed from the dorsal lobe of the mantle. The Brachiopod larva is stated not to possess the head-kidney so characteristic of ordinary Trochospheres.[1]

Shipley (41) after pointing out the importance of the absence of the head-kidney in Brachiopoda, has come to the conclusion that there is no close relationship between these animals and the Polyzoa, and further that they are not nearly allied to Phoronis. My observations do not warrant me in criticising Caldwell's statements on this head, although it does not appear to me improbable that the similarities between Brachiopoda and Phoronis may be superficial. The position of the cephalic segment with the eyes inside the mantle cavity during the metamorphosis of Argiope, seems, however, to render Caldwell's view that the præoral lobe in Brachiopoda persists in part as the epistome, as in Phoronis, not impossible.

Brooks' observations (16) on Lingula probably show that the larvæ of this form are more easily reducible to the Trochosphere type than those of the hinged Brachiopods. It appears to me that the curvature of the alimentary canal of Lingula is the same as that of a Trochosphere, and consequently directed in the opposite direction to that of Phoronis. It is hardly necessary to criticise Brooks' remarks on the similarity between the larvæ of Brachiopoda and Loxosoma, based, as they are, on what I regard as an entirely mistaken view (due to Barrois) of the archetype from which the Polyzoan larvæ are derivable.

One of the main difficulties in the comparison between the Polyzoa and other forms is the great uncertainty of the relationships of the Polyzoa *inter se*. Lankester (45) has adopted the view that Rhabdopleura and the Phylactolæmata are allied to Phoronis and consequently are more archaic than the Entoprocta; the main reason for this view is their possession of a body cavity, a structure certainly not present in the Entoprocta in the same form. From the researches of

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[1] This statement probably requires further proof.

Caldwell on Phoronis, and of Hatschek on Polygordius (17) and Echiurus (25), it appears possible that the ancestral Trochosphere-like form was provided with a well-developed body cavity, a structure represented also in some Molluscan Trochospheres. If this is the case, we must suppose that in most Trochospheres the formation of the body cavity has been postponed till after the commencement of the free existence. The Polyzoa are in this case probably descended from a Trochosphere-like organism, in which a postponement of the formation of the body cavity had become normal. It is possible that the Entoprocta have never advanced beyond this stage, whereas the Ectoprocta, in other respects more modified, have retained the habit of developing a body cavity. That this is really the interpretation of the body cavity of the Ectoprocta appears to me, however, in the highest degree doubtful, and in no known Ectoproctan ontogeny does a cœlom develop in the embryo by an enterocœl formation or by any means which can be considered a modification of this process, as in Actinotrocha.

The larvæ of the Ectoprocta are not provided, as far as is known, with a body cavity, a structure which appears first in the adult. In all probability the primary individual of the Ectoproctan colony is developed as a bud on the larva, and the wide difference in structure obtaining between the adult and the larva is quite comprehensible on this hypothesis. It seems to me that the body cavity of the Ectoprocta is a structure which is developed solely in relation with the adult condition, and that it is not directly comparable to that of other animals. The suggestion that the cœlom of Ectoprocta is a space developed between gut and body wall for the purpose of permitting the retraction of the lophophore with its tentacles into the cavity of the zoœcium appears to me not unreasonable. In the Gymnolæmata (see Vigelius, No. 46) the body cavity is a space traversed by irregular strands of connective tissue, and is as a general rule lined neither on the side of the gut nor of the body wall by an epithelial layer (Flustra). In the Phylactolæmata, how-

ever (Nitsche, 4), it is in many parts lined by a distinct ciliated epithelium. It is not impossible, however, to regard this as merely an advance on the condition found in Flustra, as the result of an increased differentiation of certain cells situated between body wall and gut, instead of supposing that it represents an archaic character, which we see in various stages of degeneration in other Polyzoa. On this hypothesis, the cilia would have arisen in order to provide a means for the circulation of the body fluids. Lankester has described a body cavity in Rhabdopleura, although it is far less pronounced than in the Gymnolæmata. The question arises, how far can Rhabdopleura and Cephalodiscus (35 and 49) be considered archaic forms? In the characters of the budding processes, these genera show a very slight amount of complication, Cephalodiscus being solitary, and producing paired buds from the end of the stalk, whilst the branching of Rhabdopleura is of a simple nature compared with that of many Ectoprocta. In certain features these forms are more modified than the Entoprocta; it is probable that the arrangement of their tentacles is less primitive than in Loxosoma; the existence of a mesoblastic skeleton (*vide* Lankester) and the character of the tubarium are again features in advance of those of the Entoprocta. The occurrence of a pair of eyes in the adult Cephalodiscus is a fact of considerable interest; the eyes are borne on an organ which is stated to be an ovary, and M'Intosh (35) suggests that this will be found to be the nature of the dorsal organ of the Entoprocta! In Rhabdopleura no retraction of the tentacles is possible, and the body cavity can hardly have the origin which has been suggested for that of the Ectoprocta.

It will be difficult to determine the relation of Rhabdopleura to the other Polyzoa until we are able to learn something of the embryology of this form.

As a result of the foregoing considerations, it appears to me that in order to understand correctly the phylogeny of the Polyzoa, we must derive the group from a Trochosphere-like organism, and that the Entoprocta have remained

permanently at a grade hardly higher than that of this hypothetical ancestor. Loxosoma shows itself the most primitive genus by the fact that it forms no colonies, by the greater development of the brain in the larva, and by the invariable presence of a foot-gland in the buds, if not in the adult. Rhabdopleura and Cephalodiscus on the one hand, and the Ectoprocta on the other, have probably branched off independently from the Entoproctan stem. In certain features the Gymnolæmata show themselves more archaic than the Phylactolæmata; this is perhaps the case with the "body cavity," whilst embryology, as far as we understand the facts, seems certainly in favour of this hypothesis. The occurrence of statoblasts, as well as the great differentiation of the funiculus, again prove that the Phylactolæmata are in some of their characters at least as much modified as the Gymnolæmata. In the structure of the nervous system, the former are, however, perhaps more archaic than the latter; in Alcyonella, Nitsche (4) describes a large suboesophageal ganglion sending off nerves to the lophophore, and connected in front of the œsophagus by a thin commissure containing, however, no ganglion cells.

The similarity in many important features between Loxosoma and a Molluscan larva has already been pointed out, and of all organisms with whose ontogeny we are acquainted, the Mollusca come nearest to the Polyzoa. The comparison between the development of the brain and pedal ganglia in Dentalium and the Hyaleacea, and the same structures in Loxosoma, may again be called attention to. The ciliated ring of the Entoprocta is very probably the velum, and the foot-gland the shell-gland. Allman's suggestion that the buccal shield of Rhabdopleura (and hence the epistome of Loxosoma) may represent the mantle area of Lamellibranchs cannot be accepted, if we are to identify the dorsal organ as the brain. If it is necessary to look for a mantle cavity in the Entoprocta, it seems to me that we may possibly find it in the vestibule. In this case the tentacles would represent, not gill filaments of Lamellibranchs, but

rather the tentacular structures developed at the edges of the mantle lobes in some of the latter.

The Rotifera, as has frequently been suggested, in many points of their structure resemble the Polyzoa, and more especially the Entoprocta: many of these similarities have already been indicated by the Hertwigs (31). Salensky (15) has drawn attention to the possible homology of the "antennæ" of Rotifers with the posterior sense organs of Loxosoma crassicauda. Histologically, the resemblances between Rotifera and Entoprocta are striking: in the characters of their nervous system, muscular fibres, terminations of the excretory organs, and so on. Perhaps the similarities between the two groups are less striking than those obtaining between the Entoprocta and the Trochosphere larvæ of Molluscs and Chætopods, but the fact remains that the Rotifera and Polyzoa being alike groups which have persisted in the Trochosphere stage, must necessarily show a considerable number of similarities to one another in various features.

The affinity between the Polyzoa and the Brachiopoda is probably much less close than that between the former and the Rotifera and the Trochospheres of Mollusca and Chætopoda, so that the association of the Polyzoa with the Brachiopoda as Molluscoidea appears to me unnatural, although I do not at all deny the possibility of certain affinities between these two groups. It is, further, not impossible that Phoronis may (through Actinotrocha) to a certain extent connect the Polyzoa with the Brachiopoda, although not in the way that Caldwell supposed.

Summary of Results.

1. Species investigated: Loxosoma crassicauda, L. pes, L. singulare (?), L. Tethyæ, and L. Leptoclini, new species. The last two alone were studied embryologically.

2. The adult Loxosoma possesses a large suboesophageal ganglion, dumb-bell-like in shape, hitherto described as some part of the generative apparatus. The ganglion is developed

from the ectodermic floor of the vestibule in the bud, and is connected with a well-developed system of peripheral nerves, ending in sense-cells bearing tactile hairs, situated on various parts of the body. The adult has no supraœsophageal ganglion.

3. The excretory organ (paired) probably begins with a flame-cell : it is composed of a few large perforated cells, their walls filled with greenish-yellow granules, and it opens into the vestibule by an aperture (on each side) between the ganglion and the œsophagus. The nephridia are completely homologous with the head-kidneys of Trochosphere larvæ.

4. No hermaphrodite individuals were discovered in any species of Loxosoma or Pedicellina. Previous statements as to the hermaphroditism of Loxosoma are partly due to an erroneous interpretation of the ganglion as the testes. It is by no means improbable, however, that the same individual develops ovaries and testes at different seasons. Schmidt's accounts of the generative organs are not correct.

5. In L. Leptoclini and in P. echinata the ova are small, and the embryo is supplied with nutriment from the glandular epithelium of the brood-pouch. In L. Tethyæ this is not the case, but the ovum is large, and during maturation devours various cells which play the part of a vitellarium.

6. After the formation of the gastrula the blastopore probably remains in the position of the anus, a stomadæum being formed anteriorly. The mesoblast arises mainly from two pole-cells situated at the sides of the blastopore.

7. The "dorsal organ" is not hypoblastic in origin ; it is formed first as a thickening, and then as an unpaired invagination of epiblast. The invagination closes, a layer of fibres is formed on its deep side, and a pair of large eyes with well-developed lenses appears on the organ. The "dorsal organ" is not a budding structure, but is the supra-œsophageal ganglion.

8. Between mouth and anus is formed a pair of invaginations of epiblast, their cavities soon fusing medianly. These, the "vestibular invaginations," remain permanently open,

their cavity widening. They form the deeper part of the vestibule, the suboesophageal ganglia arising as a pair of thickenings of their floor. The wings of the crescent-shaped brain meet the suboesophageal ganglia at the sides of the oesophagus, thereby establishing a complete circumoesophageal nervous ring.

9. After the commencement of the free life (L. Leptoclini) no fixation was observed; the larva, however, developed a pair of buds similar to those of the adult. These buds are situated at the sides of the dorsal organ, but are quite distinct from it; they are visible as epiblastic thickenings long before the embryo is ready to be hatched.

10. The stomach, before the larva becomes free, seems to bud off paired lateral bands of cells (L. Leptoclini). During the free life these increase in number, and the cells of the stomach themselves divide up during an atrophy of its lumen. This, no doubt, indicates the approaching death of the larva, after the buds have reached maturity. Some of the hypoblast cells, whose origin has been just described, probably form the endodermic tissues of the bud.

11. The Entoprocta, larval and adult, are true Trochospheres, possessing a ventral flexure of the alimentary canal, no true body cavity, and a pair of head-kidneys.

12. The nervous system of Loxosoma develops in almost exactly the same manner as that of Dentalium, in which the brain arises by invagination (paired), the pedal ganglia as thickenings of the epiblast; the connection between these two independently developed parts is secondarily set up as in Loxosoma.

13. The metamorphosis of the Ectoprocta is to be regarded as a process of budding. Cyphonautes and Tendra probably supply important evidence with regard to the derivation of the larvæ of Ectoprocta from those of Entoprocta.

14. The Entoprocta, being true Trochospheres, have certain affinities with Actinotrocha; there is every evidence that the direction of the gut-flexure differs from that of the adult

Phoronis, although it is identical with that of Actino-trocha. The line between mouth and anus is ventral in Polyzoa, but dorsal in Phoronis.

15. The affinity of the Polyzoa to the Brachiopoda is probably more doubtful than to Phoronis, the larvæ of the two groups differing in many important characters (*e. g.* the absence (?) of head-kidneys in the larval Brachiopod).

16. The nearest allies of the Polyzoa (*i.e.* of the Ento-procta) are the Trochosphere larvæ of Mollusca or Chætopoda and the adult Rotifera.

17. The Entoprocta form the most archaic of the groups of the Polyzoa, their relations to the other forms being, however, somewhat doubtful.

Note.—Fewkes (42) has recently described a new larva, belonging to an unknown adult, and regarded by him as being of great importance in forming a connecting link between Polyzoa and Annelids. In spite of the assertion of Fewkes that "there is no doubt that it is a larval Annelid," I venture to believe that it has no importance in the sense ascribed to it by the American zoologist, and that the animal in question is simply a very characteristic larval Loxosoma. The relations of the ciliated ring in Fewkes' larva are precisely those of the Entoprocta; there are two eyes, no doubt indicating the presence of a dorsal organ (whose cilia are described); the anus opens on an anal cone; the stomach has orange granules, the situation of the mouth not having been observed with complete certainty, although believed to occur just within the ciliary ring, which can be reflected over the oral face of the larva.

These features, together with the figures given, leave no doubt in my mind that the larva described by Fewkes is merely that of a Loxosoma.

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EXPLANATION OF PLATES XIX—XXI,

Illustrating Mr. S. F. Harmer's Paper " On the Structure and Development of Loxosoma."

Reference Letters.

ac. Cells at the apex of the vestibule in L. Leptoclini. *afg.* Aperture of foot-gland. *agc.* Aperture of gland-cell. *al.* Lateral expansion of foot. *an.* Anus. *b.* Bud. *bl.* Blastopore. *blc.* Blastocœl. *br.* "Dorsal organ " (= Brain). *cd.* Ciliated duct of nephridium. *cr.* Ciliated ring. *ct.* Connective-tissue cell. *ctp.* Large cells in tentacles of Pedicellina. *cut.* Cuticle. *ean.* External aperture of nephridium. *ebp.* Glandular epithelium

of brood pouch. *el.* Epithelial cell of ectoderm. *em.* Embryo. *ep.* Epiblast. *epst.* Epistome. *f.* Foot. *fbr.* Fibrous part of brain (or "dorsal organ"). *fg.* Foot-gland. *fgd.* "Duct" of foot-gland. *fl.* Flagellum of flame-cell. *ga.* Ganglion of adult. *gac.* Ganglion-cell. *gas.* Ganglion of posterior sense organ of L. crassicauda. *gc, gc¹,* and *gc².* Gland-cell. *hst.* Hypoblastic outgrowth of stomach. *hyp.* Hypoblast. *I.* Parasitic Infusorian. *int.* Intestine. *l.* "Liver-cells" of stomach. *lst.* Lateral wing of stomach. *m.* Mouth. *mc.* Mantle cavity. *mes.* Mesoblast. *mus.* Muscle-cell. *n.* Nucleus. *nv.* Nerve. *o.* Eye. *œ.* Œsophagus. *œc.* Œsophageal commissure. *og.* Oral groove. *ot.* Position of ovary or testis. *ov.* Ovary. *ovd.* Oviduct. *p.* External aperture of female generative organs. *pm.* Pole-cell of mesoblast. *ps.* Posterior sense organ of L. crassicauda. *rec.* Rectum. *s.* Sucker. *sc.* Sense-cell. *sg.* Position of subœsophageal ganglion of embryo. *st.* Stomach. *std.* Stomodæum. *t.* Tentacle. *tes.* Testis. *tga.* Tentacular ganglion. *v.* Vestibule, or vestibular invagination of embryo. *va.* Aperture of vestibule. *vd.* Vas deferens. *vm.* Vitelline membrane. *vs.* Vesicula seminalis. *vt.* Vitelline body in ovum. *z.* Junction of stomodæum and mesenteron of embryo.

PLATE XIX.

Fig. 1.—Loxosoma crassicauda. Calyx, seen from the posterior side (living) to show the nervous system; the cilia of the tentacles are not represented. The figure is combined from numerous sketches of the different parts of the nervous system, made from living specimens or from glycerine preparations. The individual represented has neither buds nor generative organs. *st.* Stomach. *ga.* Ganglion. A full explanation of the figure is given in the text.

Fig. 2.—L. Leptoclini (new species). An entire animal, from the anterior side.

Fig. 3.—L. crassicauda. The whole of the posterior ectoderm cells of the calyx, after treatment with silver nitrate. *ps.* One of the posterior sense organs.

Fig. 4.—L. crassicauda. A posterior sense organ, after the action of silver nitrate, osmic acid, and picro-carmine. *ps.* is the actual sense cell, situated at the apex of a rounded papilla formed by three ordinary ectoderm cells. (From a nearly mature bud.)

Fig. 5.—Pedicellina echinata. A portion of a tentacle, from a preparation treated with osmic acid and picro-carmine and mounted in glycerine. The tentacle is seen in optical section. *el.* outer ectoderm of the tentacle.

Fig. 6.—Loxosoma pes. A sense cell in connection with two ganglion-cells. (From a glycerine preparation.)

Fig. 7.—L. crassicauda. A portion of the edge of the calyx (silver nitrate, osmic acid, and picro-carmine), showing the apertures (*agc.*) of the

gland-cells, and two ganglion-cells (*gac.*) in connection with their respective sense cells (*sc.*).

FIG. 8.—L. crassicauda. Part of the edge of the calyx (living) in optical section.

FIG. 9.—L. Tethyæ. A portion of the stalk, from a glycerine preparation, previously treated with palladium chloride. The upper half of the figure shows the ectoderm in surface view, the lower half representing an optical section of one of the rows of cells.

FIG. 10.—L. crassicauda. Connective-tissue cells, from the side of the stomach. (From a glycerine preparation.)

PLATE XX.

FIG. 11.—L. crassicauda. A median longitudinal section of an entire individual; only the ventral end of the stalk is involved by the section, which passes through the whole of the alimentary canal, through the ganglion (*ga.*), and two of the tentacles (*t.*). At the base of each of the latter, on its outer side, is seen a small accumulation of nuclei (ectodermic), representing all that can be made out in the section of the crumpled edge of the vestibular fold, which during retraction of the tentacles completely encloses the vestibular cavity. The animal was killed, during extension of the tentacles, by boiling corrosive sublimate (a saturated solution in sea water).

FIG. 12.—Pedicellina echinata. A horizontal section of a female containing embryos (*em.*) in the brood pouch, which is lined by a glandular epithelium (*ebp.*) thrown into numerous folds. The œsophagus (*œ.*) and intestine (*int.*), the ganglion (*ga.*) and the ovaries (*ov.*) with their ducts (*ovd.*) are also represented. The figure was combined from two series of sections.

FIG. 13.—Loxosoma Tethyæ. A horizontal section through a male individual, killed with the tentacles extended in boiling sublimate. *tes.* Testis. *vd.* Vas deferens. *vs.* Vesicula seminalis.

FIG. 14.—L. Tethyæ. Horizontal section of a female, with tentacles retracted (the figure is combined). Ganglion (*ga.*), ovaries (*ov.*), and oviducts (*ovd.*) are represented. In the left ovary is indicated the process of the absorption of several of the primordial ova by the developing ovum. In addition to this mode of nutrition, the ovum on each side is devouring a vitelline body (*vt.*).

FIG. 15.—L. Leptoclini. An advanced bud in nearly median longitudinal section. The ganglion (*ga.*) is still in intimate connection with the vestibular diverticulum from which it has been formed.

FIG. 16.—L. Tethyae. A horizontal section of an adult, passing through the ganglion (*ga.*), the vesicula seminalis (*vs.*), and the wide mouth (*m.*), opening into the vestibule (*v.*). In this individual the testes appear to have atrophied.

Fig. 17.—L. crassicauda. A nephridium, in the living condition, very highly magnified (Zeiss, $\frac{1}{12}$, No. 5 eye-piece). The adult from which the figure is taken was in the same relative position as that represented in Fig. 2, and the'nephridium corresponding to the right side of the figure was drawn; the lower end of Fig. 17, containing the flame-cell (*fl.*) should lie on the ventral side of the stomach, the upper end (*ean.*) opening into the vestibule, the median plane of the entire animal being situated to the left of the figure.

Fig. 18.—L. crassicauda. Horizontal section of an adult, through the epistone (*epst.*), the mouth (*m.*), and the bases of some of the tentacles (*t.*). Only the basal portion of the bud (*b.*) on the left side has been represented.

Fig. 19.—L. crassicauda. Surface view of the foot-gland of a young bud, from a glycerine preparation. The constriction of the groove probably indicates the separation from one another of the "gland" and the "duct."

Fig. 20.—L. Leptoclini. Transverse section through the middle region of the foot of an adult. *al.* Lateral expansions of the foot (cf. Fig. 2). *fgd.* The "duct" of the foot-gland, in reality an open groove.

Fig. 21.—L. Leptoclini. Obliquely longitudinal section through the foot, passing through the "gland" (*fg.*), but missing the "duct."

Fig. 22.—Tendra zostericola, copied from Repiachoff (27), plate ii, fig. 5 (a median longitudinal section of an embryo). Probable explanation of Repiachoff's letters:—*o.* Mouth. *v.* Vestibular invagination (cf. fig. 39). *g.* Stomach. *y.* Sucker or foot-gland. *e.* hypoblastic cells taking part in the formation of the first bud. *x.* dorsal organ (?)

Fig. 23.—Dentalium. Transverse section of a young individual (copied from Kowalevsky, No. 36 in the list of references, pl. viii, fig. 92). *br.* Brain. *œ.* œsophagus. *mc.* Mantle cavity. *f.* Foot.

Fig. 24.—Cyphonautes. An entire larva (slightly modified from Repiachoff, No. 27, plate i, fig. 1). Probable explanation of the lettering:—*a.* Vestibule. *œ.* Œsophagus. *y.* Sucker. *g.* Stomach (containing food particles). *r.* Rectum. *zc.* Dorsal organ (?). *x.* Epiblastic portion of young bud. *e.* Hypoblastic portion of the bud.

PLATE XXI.

With the exception of figs. 27 and 55, all the figures represent actual sections illustrating the embryology of Loxosoma. Figs. 25—59 belong to L. Leptoclini; Figs. 60—62 to L. Tethyæ. The sections are all drawn to the same scale, with the exception of Figs. 54, and 56—59, which are more magnified.

L. Leptoclini:

Fig. 25.—Ovarian ovum.

Fig. 26.—Two-cell stage.

Fig. 27.—Drawn living, as seen through the walls of the vestibule of the adult. *vm.* Vitelline membrane.

Fig. 28.—A section of an embryo consisting of about eight cells.

Fig. 29.—Blastosphere stage. *blc.* Blastocœl.

Fig. 30.—Commencement of the invagination; the blastocœl is almost obliterated.

Fig. 31.—The invagination has progressed to a considerable extent, but the blastocœl is much larger than in Fig. 30.

Fig. 32.—A slightly older embryo. *pm.* Pole-cell of the mesoblast.

Fig. 33.—A similar stage, the section having missed the blastopore and the pole-cells.

Fig. 34.—The invagination is here far advanced, the blastocœl obliterated. The blastopore appears to be filled up by one of the pole-cells, which really, however, lies in a somewhat deeper plane of the section.

Fig. 35.—A nearly median longitudinal section of a stage after the formation of the stomodæum, whose position in a neighbouring section is indicated by the line *m.* The ventral gut flexure is already acquired; at the posterior end are two mesoblast cells.

Fig. 36.—Another section of the same embryo. *v.* is the commencement of one of the vestibular invaginations; *og.* is the oral groove. The cells which fill up part of the blastocœl belong in part to one of the lateral walls of the stomach.

Fig. 37.—A more advanced embryo in median longitudinal section. *z.* Junction of stomodæum and mesenteron. *s.* The sucker. *br.* The epiblastic thickening forming the dorsal organ or brain.

Fig. 38.—A stage slightly further advanced in development. The brain (*br.*) consists of two layers of cells (neither this section nor Fig. 37 has passed through the intestine).

Fig. 39.—Obliquely longitudinal section of an older embryo, cutting the œsophagus (*œ.*), stomach (*st.*), sucker (*s.*), brain (*br.*), and one of the vestibular invaginations (*v.*).

Fig. 40.—More advanced than Fig. 39. The section passes at some distance from the middle line, but parallel to the symmetrical plane of the embryo. *og.* Oral groove. *v.* Vestibular invagination. *br.* One of the lateral wings of the brain (compare with fig. 46). *st.* Wall of the stomach. *mes.* Mesoblastic band. The blastocœl contains in addition one or two mesoblast cells which in the section do not appear connected with the mesoblastic band.

Fig. 41.—A somewhat similar section, passing obliquely in such a manner as to cut only the more ventral regions of the embryo, and thereby to avoid the stomach. The blastocœl contains numerous mesoblast cells. *mus.* is a muscle-cell, branched at one end.

Fig. 42.—Another section of the same embryo avoiding the brain, vestibule,

tga

tga

nv

sc

gac

rec

int

gas

ps

ga

1

nv
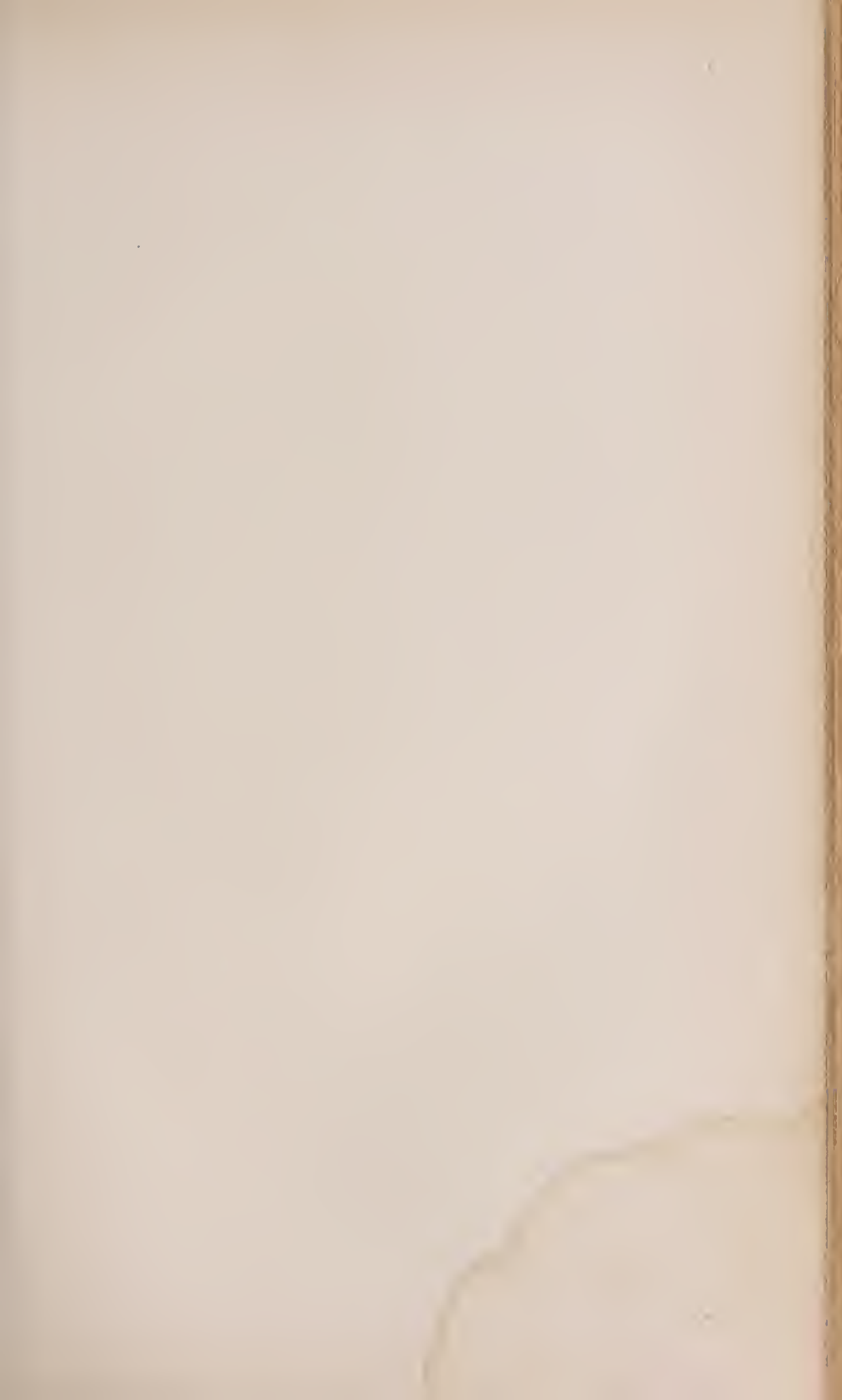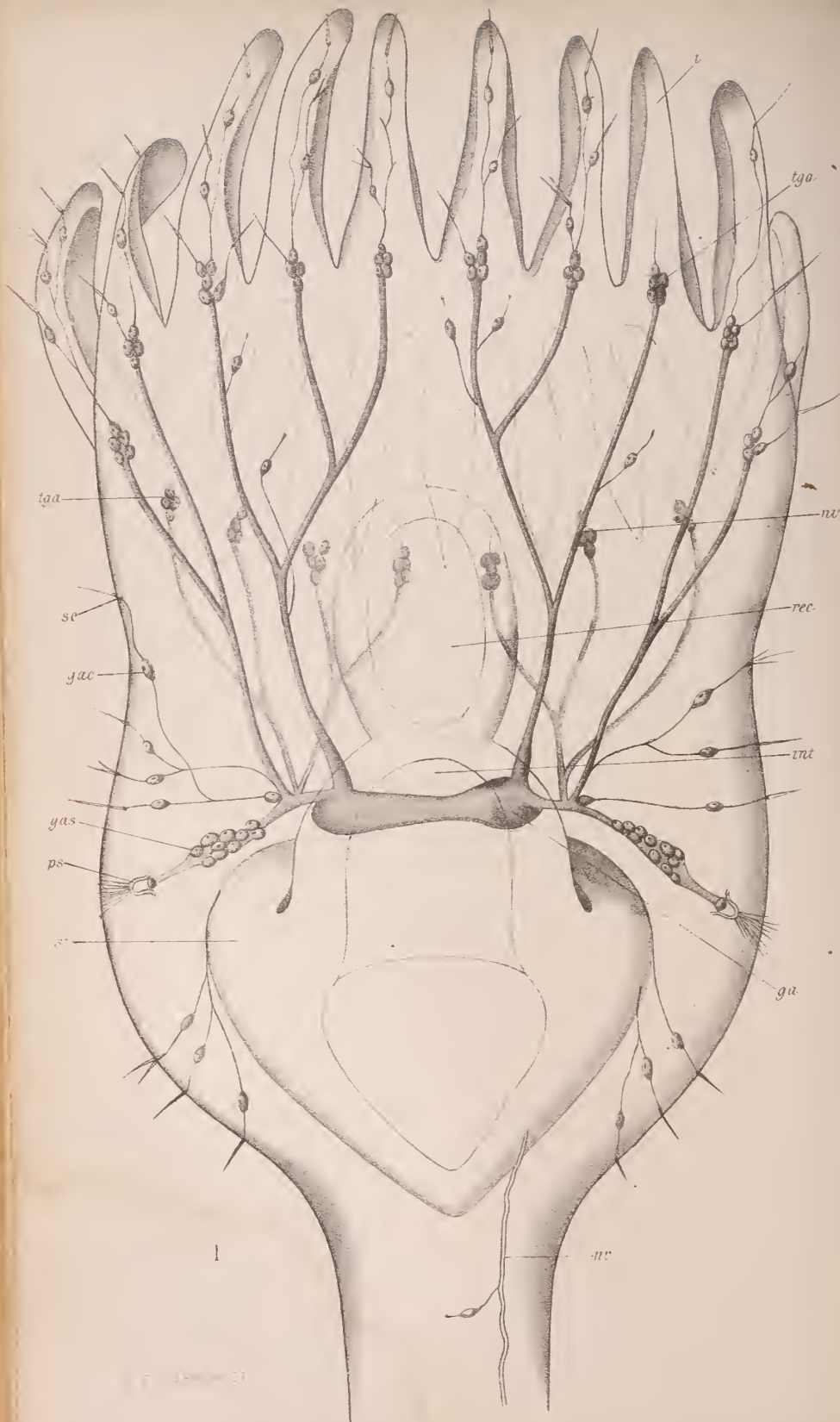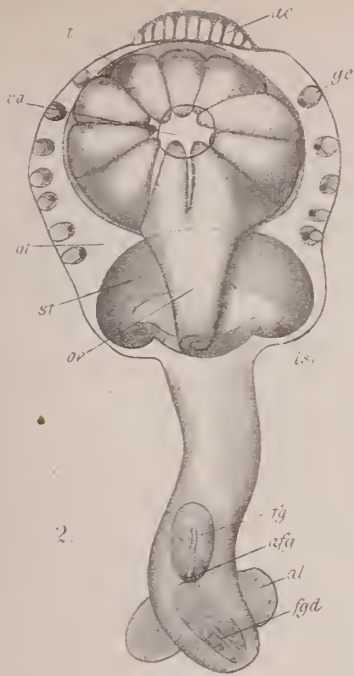
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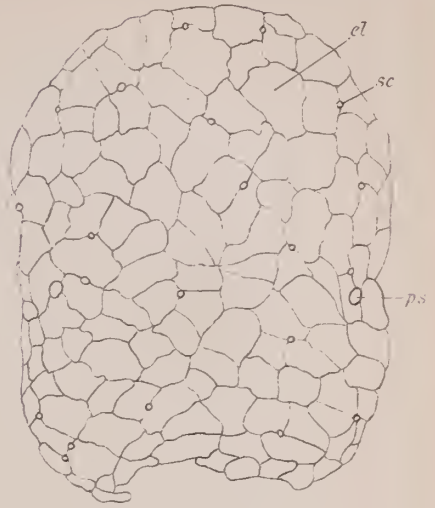
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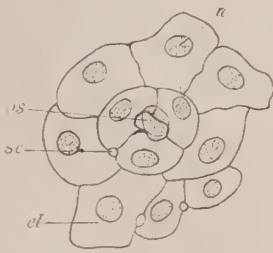
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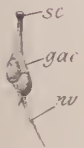
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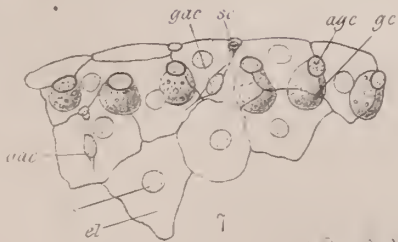
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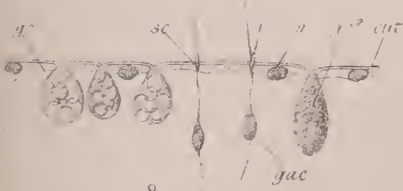
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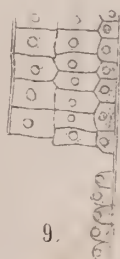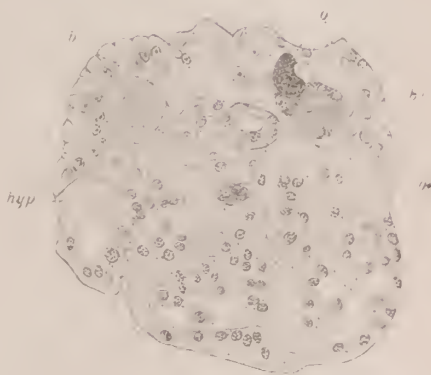
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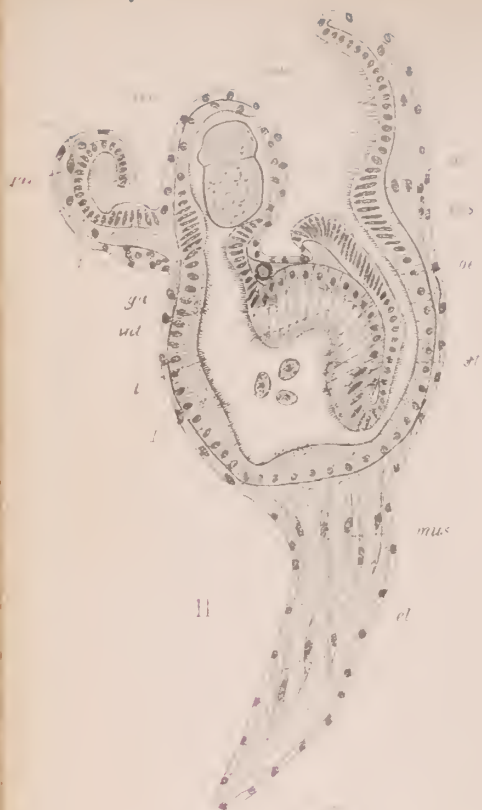
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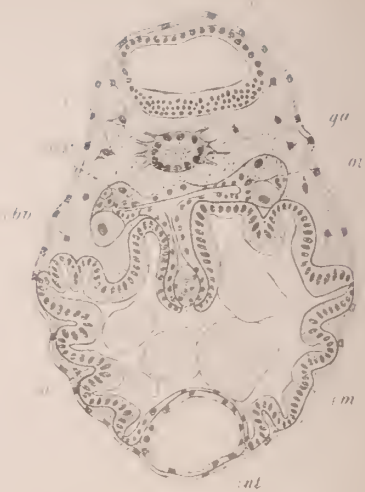
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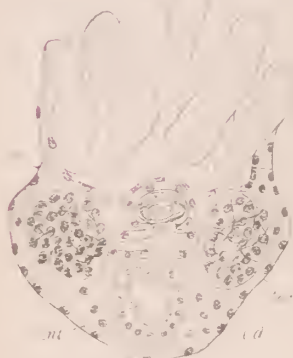
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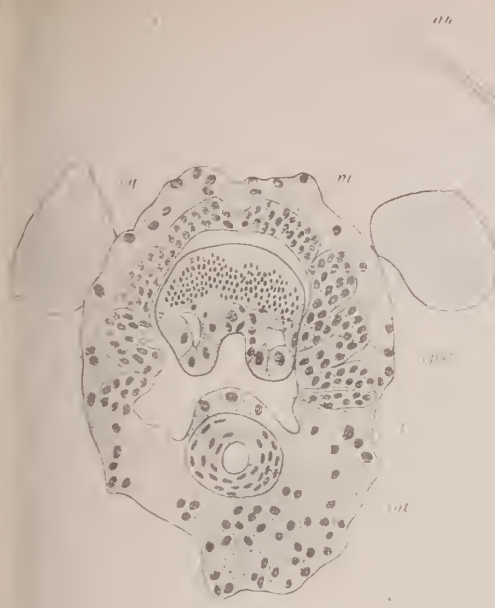
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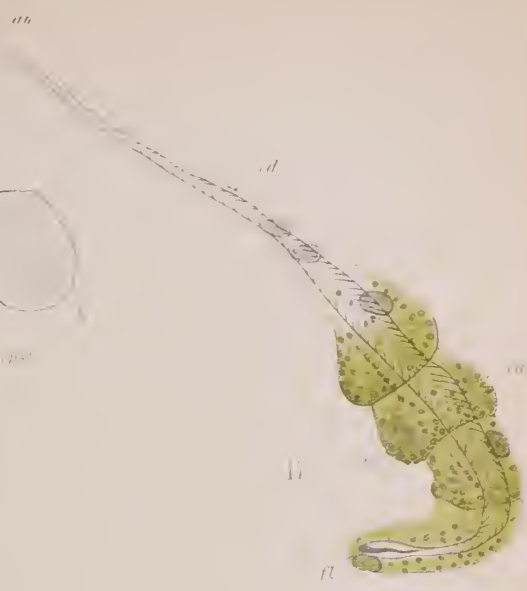
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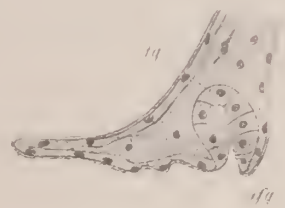
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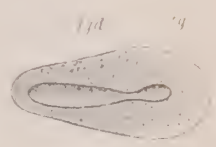
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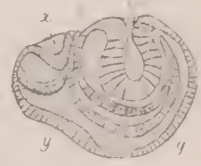
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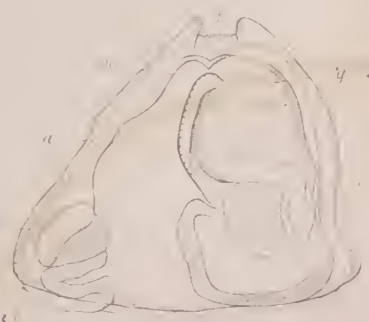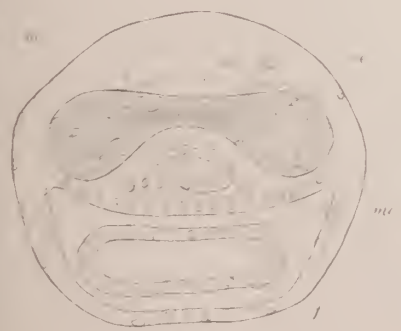
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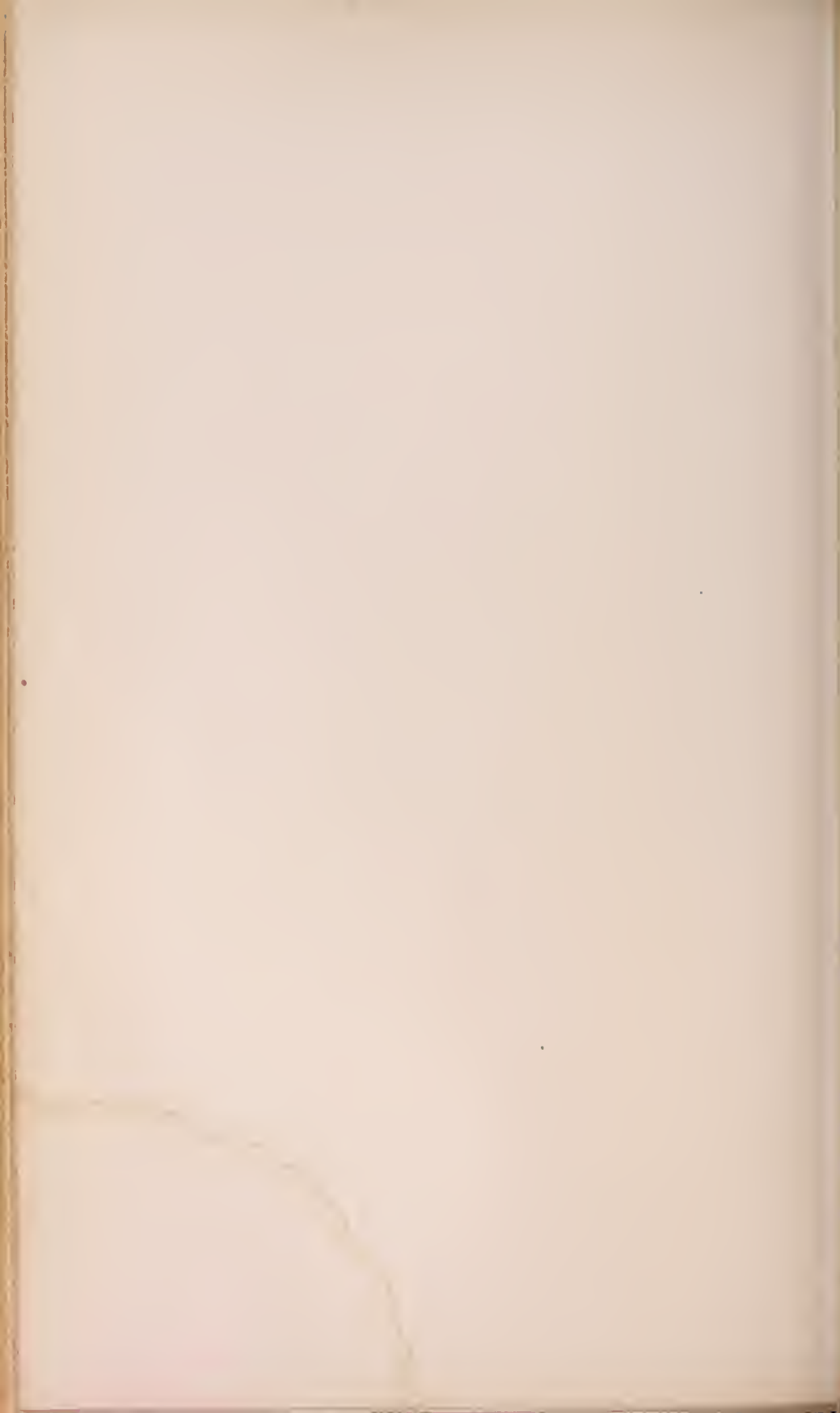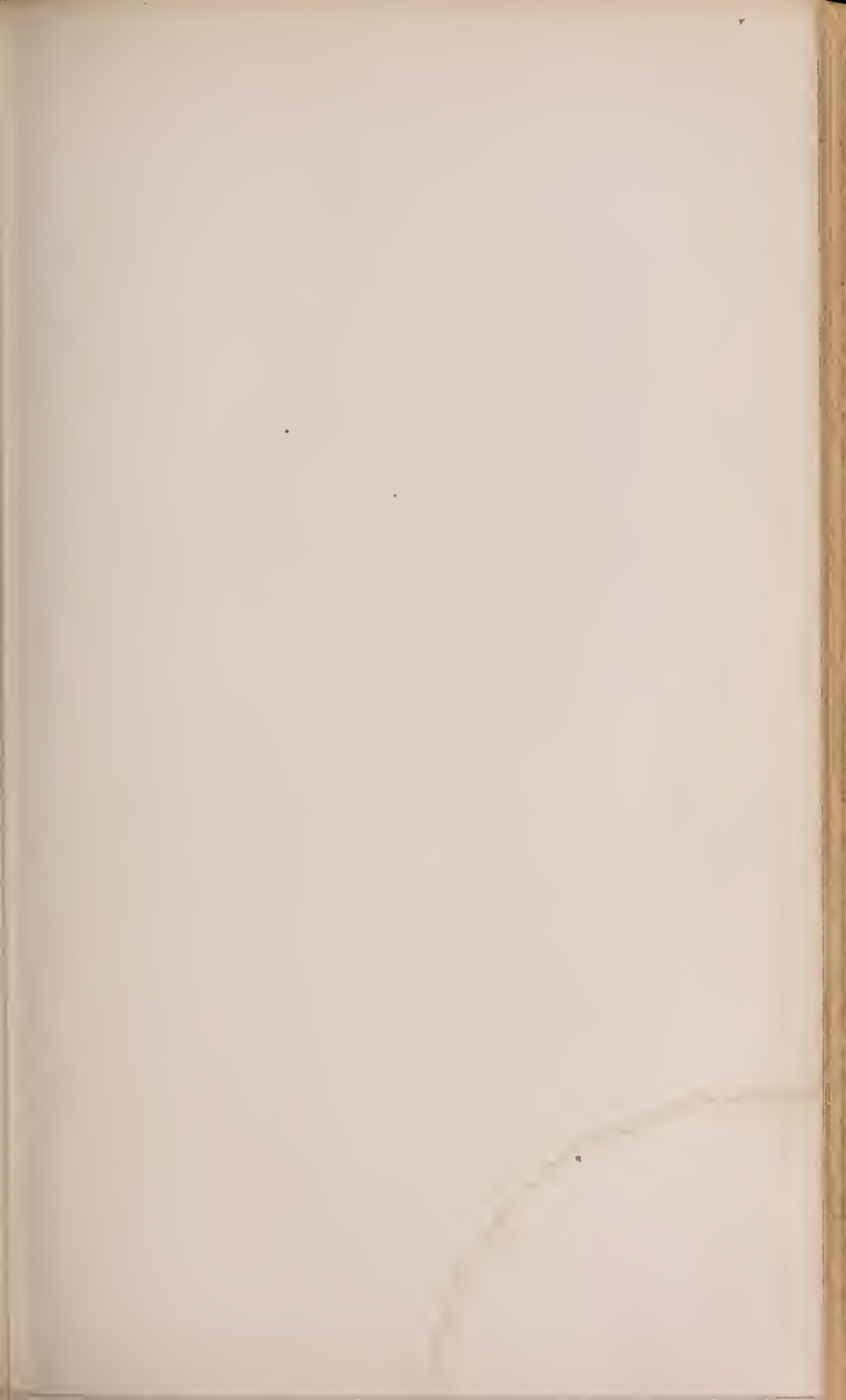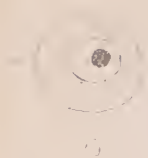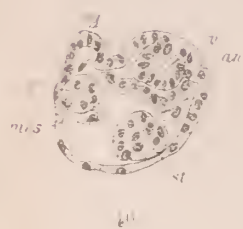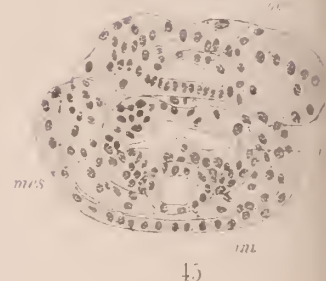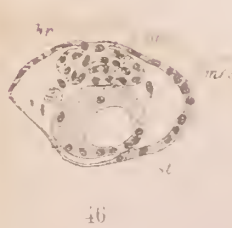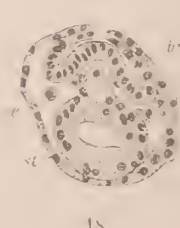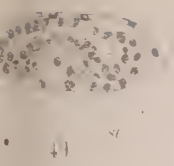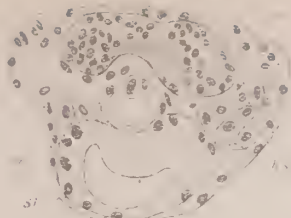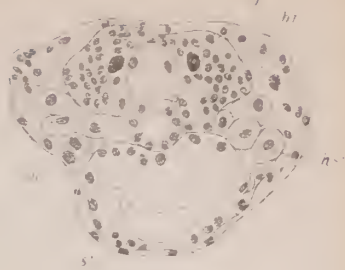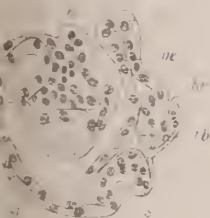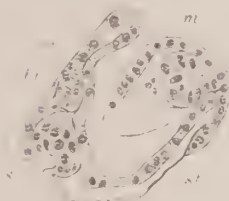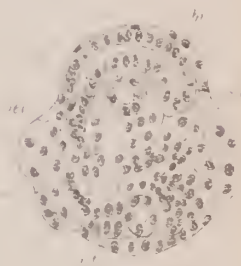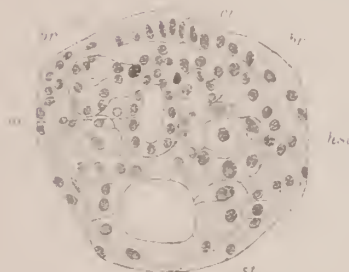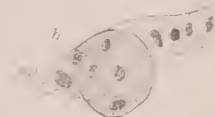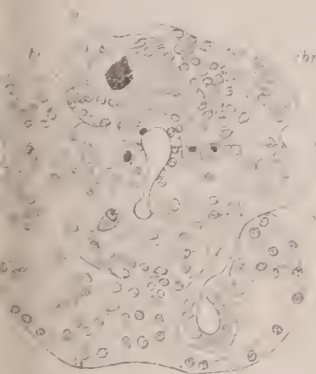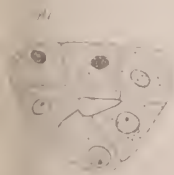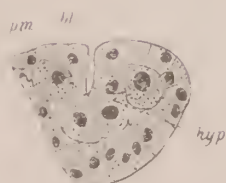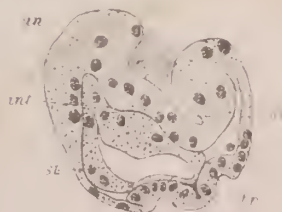
A New Hypothesis as to the Relationship of the Lung-book of Scorpio to the Gill-book of Limulus.

By

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THE view which I advocated in my essay 'Limulus an Arachnid,' as to the mode of conversion of an external lamelligerous appendage into the hollow lamelliginous lung of Scorpio no longer commends itself to me. A much simpler and, as it appears to me, a thoroughly satisfactory explanation of the relationship of the two organs, has occurred to me in the course of recent investigations, and is supported also by embryological data. In the essay above referred to I suggested that by the enlargement of the hollow stigmata connected with the thoraco-branchial muscles of an ancestral Scorpion, resembling Limulus in having branchigerous appendages on the mesosoma, and provided with thoraco-branchial muscles, the branchigerous appendage might come to lie in the pit or hollow of the tendon of the thoraco-branchial muscle, and eventually the hollow might enclose it. The conversion of the insunken appendage into a hollow air-holding sac and the corresponding conversion of the surrounding pit into a closed blood-holding space, involved serious difficulties which were indeed fatal to the hypothesis.

When I found ('Transact. Zool. Soc.,' vol. xi, part x, 1884) that the muscle (veno-pericardiac) attached to the apex of each lung-sinus in Scorpio had no possible relation to the thoraco-branchial muscles of Limulus, but was represented in Limulus by exactly similar veno-pericardiac muscles, I gave

up my over-strained hypothesis. I trust that the failure of my previous suggestion will not unduly prejudice those interested in this subject against that which I now advance.

Since my memoir ' Limulus an Arachnid,' Dr. MacLeod, of Brussels, has published some speculations on this subject, in which he puts forward an ingenious theory of his own as to the mode in which the lamelligerous appendage of a Limulus-like animal might be converted into the lamelligerous lung-book of an Arachnid. I will not enter into a discussion of Dr. MacLeod's hypothesis, but will merely point out that inasmuch as it deals with not the less modified lung-book of Scorpion, but the more modified lung-book of the Araneina, it is unsatisfactorily elaborated. The lung-book of Scorpio has a definite axis carrying the leaf-like lamellæ, and corresponding to the axis of the same animal's pecten. Such an axis is not present in the Araneine lung-book, and yet must be accounted for as a primary structure in any theory as to the origin of these organs.

The hypothesis which I now put forward is perfectly simple, and leaves, I think, nothing to be desired. In Limulus, as in Scorpio, there is on each side of the sternal surface a great blood-sinus in free communication with the lamelligerous organs. Let us suppose such to have been the case in the common ancestor of these two animals, and let us suppose that this ancestor possessed six pairs of mesosomatic appendages, of which five were lamelligerous and intermediate in form between the pectens of Scorpion and the recent Limulus appendage. Now, suppose that in the Scorpion branch of the family the mesosomatic appendages grew relatively smaller and smaller, were no longer locomotor organs, but purely respiratory, and served for aerial rather than aquatic respiration. If we imagine the four hinder pairs of these reduced appendages to have taken on in the embryonic condition a very common trick of growth, viz. an inward growth of invagination, so that they grew into the Scorpion's body, turning their outside in, just as a glove may have all its fingers and part of the hand turned outside in—then we should have without further alte-

ration the exact condition of the modern Scorpion's lung-book. The appendages growing thus inwards by introversion (instead of outwards, as is normal) would simply be tucked or pushed into the great blood-sinus, which would constitute around each in-grown appendage a veinous sac, just as we actually find in the Scorpion. The most familiar case of inward growth taking the place of outward growth is in the development of the Tænia-head upon the cyst of the hydatid in such a form as T. solium. The head develops in a perfectly normal way, excepting that it is completely introverted, pushed outside in, and at a certain stage it becomes everted, as it should have been from the first, had it retained in growth its ancestral relations. The cause of the introverted growth of the Tænia-head on its cyst is very probably external pressure ; in fact, the growing mass of tissue takes the direction of least resistance, and grows into the cyst instead of out from it. It is not at all improbable that such a condition of external pressure might in the first instance have induced the inward growth, during development, of the lung-books of the Scorpion. The development of the young Scorpion goes on at the present day under very remarkable conditions, actually in the ovary, the egg-cell never moving from its place of origin until it has grown into the fully-formed Scorpion ; the pressure of the ovarian tunic upon the surface of the growing embryo must be considerable, and is at any rate a possible cause of the invagination of the four hindermost pairs of mesosomatic appendages in the first instance. Probably the lamelligerous appendages of the young Scorpions, of a certain stage in the ancestry of recent Scorpions, were everted and assumed the normal relations of appendages as external processes of the body-wall as soon as the young were born. But as the lamelligerous appendages were only required to act as aerial respiratory organs it would be no disadvantage, but positively an advantage, that they should remain in the introverted condition ; and this at last has become the permanent condition. This hypothesis accounts for the fact that the four pairs of lung-books do not ever appear on the surface of the embryo Scorpion as

up-standing appendages. They are from the first introverted, and remain so. It also agrees with the disposition of the cuticularised surfaces of the Scorpion's lung-book, as seen in the adult. The cuticularised surface remains in the in-pushed as it is in the out-growing appendage, the surface in contact with the air. Each bag-like lamella is introverted together with the axis of the limb; and one cannot better picture to oneself the relative conditions of out-growth and in-growth than by fixing a kid glove by the margin of its opening to the margin of an opening of the same size on the outside of a box. The coloured surface of the kid will represent the cuticle, the fingers the lamellæ, the hand the axis. Thus the glove will represent a lamelligerous appendage, standing up on the ventral surface of an Arthropod, its cavity communicating with the cavity of the venous sinus of the animal, as the cavity of the glove does with that of the box.

Now, without removing the glove, push all the fingers from their tips inwards into the hand, and then the hand into the box, so as completely to turn the glove outside in. Thus the glove will represent the appendage when introverted into the veinous sinus as in the modern Scorpions.

The tips of some of the introverted lamellæ of the Scorpion's gill-book have aquired laterally, but not in every part, an attachment to the wall of the veinous sac into which they have pushed their way. These attachments and the relation of blood-space, air-space, and cuticle in the lung-lamellæ of Scorpio are figured in the 'Trans. Zool. Soc.,' vol. xl, 1885.

On Spermatogenesis in the Rat.

By

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With Plates XXII and XXIII.

THE following paper is the result of an investigation which
I have made during the past year, in the Physiological Labo-
ratory at University College, upon the spermatogenesis of the
Rat, which, owing to the large size of its spermatozoa, and
especially of the " middle piece," presents peculiar advantages
for the purpose. Although minor points of difference exist
(in the relative length of the " middle piece " of the sper-
matozoon and in the shape of the " head ") the process is
apparently in all essential points identical with that which
occurs in other mammals, and hence may be taken as a type
of " mammalian spermatogenesis."

Methods of Research.—I have studied the process of
spermatogenesis by means of sections, and by teased prepara-
tions.

(1) Sections prepared by the paraffin-shellac method from
the testis gradually hardened in alcohol, i. e. by being placed
in alcoholic solution of gradually increasing strength. The
sections were prepared from small pieces of the organ, stained
in bulk, by immersion in Kleinenberg's alcoholic hæmatoxylin
solution for about three hours, the excess of stain being removed
by acidulated alcohol, or by prolonged immersion (for a week)
in a very dilute watery solution. From sections stained with

hæmatoxylin the history of the process and the structure of the nuclei can be made out.

(2) Sections stained with gold. Small pieces of the fresh testis were soaked in chloride of gold solution, 1 per cent., for one hour, after previous immersion in lemon-juice or dilute formic acid, and exposed to the direct sunlight of midsummer in distilled water acidulated with acetic acid, until the reduction of the gold was complete. Then the pieces of tissue were carefully and gradually dehydrated with alcohol, and sections prepared by the paraffin-shellac method. These preparations show in particular the protoplasmic structures and the outlines of the cells, the nuclei being entirely unstained and appearing like vacuoles; thus they present a marked contrast to the hæmatoxylin-stained sections.

(3) Sections stained with osmic acid, prepared by soaking small pieces of the fresh testis in osmic acid solution, 1 per cent., for two days, washing with water, and dehydrating carefully with alcohol. Then the sections were prepared by the paraffin-shellac method, and mounted in balsam.

(4) Teased osmic acid preparations. Small pieces of fresh testis were soaked in osmic acid solution 1 per cent. for two days, and small pieces of tubules were broken up with needles in glycerine and water.

From these teased preparations the development of the spermatozoa themselves can be best studied.

Nomenclature.[1]—I shall first describe the different cell-elements contained in a tubule, and give them the names which I propose to adopt in this account, and then trace out what I consider to be the history of the development of the spermatozoa.

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[1] The question of nomenclature presents some difficulty, since so many different names have been given to the same elements by different observers, which leads to confusion in the mind of the reader; consequently I have, at the suggestion of Professor Lankester, avoided the use of such general terms as " spermatoblast," "spermatocyte," &c., and have substituted simple descriptive expressions, descriptive of the appearance or function of the elements of the tubule. I may here mention that I am much indebted to Professors Schäfer and Lankester for their kindness in offering suggestions and advice.

In a section stained with hæmatoxylin tubules are seen, cut more or less transversely, and presenting different appearances according to the stage of development of their contents. Since the process of development of spermatozoa is a continuous one, it would be possible to take any one stage as a starting-point for description; but it is, on the whole, more convenient to start with the stage represented in fig. 1, in which a crop of spermatozoa is fully formed and ready to leave the tubule.

A tubule in this stage (fig. 1) consists of four layers of seminal elements, with a basement membrane formed of flattened cells, and these four layers correspond to four generations.

The most external layer immediately within the basement membrane consists of cells, the nuclei of which are all in the resting condition.

Of these nuclei there are three kinds represented in fig. 1 corresponding to three classes of cells.

(1) There are large pale nuclei, the diameter of which is about 18 μ, each containing a distinct round nucleolus, and being bounded by a definite nuclear membrane (fig. 1, e). In this stage some of these nuclei are seen resting upon the basement membrane, while others are seen extending between the cells of the second layer, and here and there one is found among the cells of the third layer. These nuclei belong to supporting cells, each of which serves for the support of a group of spermatozoa during their development. The protoplasm of these cells forms a sort of network upon the basement membrane, in the meshes of which the other cells of the outer layer are contained, while from the inner extremity of the nucleus a protoplasmic process extends towards the spermatozoa in the lumen of the tube. These cells appear to be constant among Vertebrata; they have received various names from different investigators, and have had very different functions assigned to them.

(2) Besides these supporting cells there are a considerable number of small cells containing oval nuclei, which stain

darkly with hæmatoxylin and present the ordinary appearance
of resting nuclei; they contain an irregular coarse chromatic
network embedded in a stroma which stains less darkly, and
are bounded by a nuclear membrane (fig. 1, *b*).   These I shall
call g r o w i n g c e l l s.

(3) The other cells in the outer layer, which might easily
be overlooked, are much fewer in number than those last-
mentioned, and their nuclei are larger, somewhat paler, and
more homogeneous in appearance, the chromatic substance
being diffused in a very fine network throughout the nucleus
(fig. 1, *a*).   These appear to me to be the cells from which all
the other elements of the tubule, with the exception of the
supporting cells, are derived, and I shall therefore call them
s p o r e - c e l l s.   Probably they are the direct descendants of the
primitive male ova.

The cells of the second layer are large, and contain large
spherical nuclei, which are all in the kinetic condition.   At
this stage the nuclei form for the most part a single row, but
here and there a cell is seen containing two nuclei, one being
internal to the other.   These correspond to the growing cells
of the outer layer of the preceding generation, which have
now attained a considerable size.   They are destined to divide
by karyokinesis into groups of cells which ultimately become
spermatozoa.   They are in fact growing cells which have nearly
finished growing.

The third layer (fig. 1, *c*) is composed of smaller cells, three
or four deep, with spherical nuclei which are pale, containing
only a small amount of chromatin.   These cells have been
called spermatoblasts, spermatocytes, &c., but since each cell
is destined to develop into a spermatazoon, I shall call them
simply y o u n g s p e r m a t o z o a, or y o u n g s p e r m a t o z o a
w i t h s p h e r i c a l n u c l e i.

The fourth layer is composed of spermatozoa which are just
ready to leave the tubule.   Between the heads of the sperma-
tozoa and the cells of the third layer, there are numerous
irregular darkly-stained granules[1] (fig. 1, *x*), each granule

[1] These bodies are the "seminal granules" which have been so often

being surrounded by a small amount of protoplasmic material. The dark granules are not derived from the destruction of nuclei, but make their appearance in the protoplasm of the developing spermatozoa at a certain stage of their development, and are cast off, together with a small amount of unaltered protoplasm, when the development is nearly completed. They appear to consist of a mixture of albuminous and fatty material, since in osmic acid preparations their place is taken by a cluster of minute black granules (fig. 15).

A very different appearance is presented by sections prepared by the chloride of gold method (figs. 12, 13, 14). A stage of development corresponding to fig. 1 is represented by fig. 12; in this preparation the nuclei are entirely unstained, and resemble clear vacuoles, but the protoplasmic structures and cell-outlines are rendered very conspicuous. The large growing cells of the second layer are seen to contain large granules—the "accessory corpuscles" of Renson—which are darkly stained by the reagent (fig. 12, b''), and in the small cells of the outer layer similar but smaller bodies are seen. The young spermatozoa which form the third layer, also each contain an accessory corpuscle, which at this stage is embedded in the protoplasm at the inner part of the cell (fig. 12, c). The fully formed spermatozoa (fig. 12, d) show an obvious division into three parts, head, body or middle piece, and tail. The tail is entirely unstained, but the middle piece contains a spiral filament which is darkly stained, and consequently very conspicuous. It winds in a close spiral round a slightly tinted core, which is continuous with the tail of the spermatozoon.

noticed; they are produced, not by the destruction of nuclei, but from the protoplasm of the developing spermatozoa, from which they separate off at a late stage of their development. They are of great interest, since they appear to represent the polar globules of the ovum, and their separation possibly means the separation of the female element from the spermatozoa. They are, I believe, constant in mammalia; although owing to the shortness of the middle piece of the spermatozoon in most animals e.g. the dog, rabbit, man, their separation cannot be made out with such distinctness as in the case of the rat.

Dr. Heneage Gibbes[1] has observed a spiral filament in the spermatozoa of several animals, e. g. horse, guineapig, and bull, which presents a somewhat similar appearance to that which I have just described; he considered this to correspond to the so-called spiral filament of the newt's spermatozoon, which is an undulating filament attached to the spermatozoon by a fine membrane, which acts as a mesentery; but it is difficult to see much resemblance between the spiral filament which is stained by the gold method, and is confined to the middle piece of the spermatozoon, and the long undulating filament of the spermatozoon of the newt. I have not yet looked for this spiral filament in the middle piece of the spermatozoa of other animals, but will endeavour to do so during the present summer.

History of the Process of Spermatogenesis.—Having thus briefly described the various elements of the seminal tubule, I shall proceed to trace out continuously the history of the origin and development of the spermatozoa.

The process is a continuous one, i. e. a new crop of cells makes its appearance near the basement membrane as each successive crop of ripe spermatozoa leaves the tubule; and in a section of a single tubule four or five generations of cells at different stages of their development are contained, so that the entire process of spermatogenesis occupies a corresponding number of cycles. An entire cycle is represented by figs. 1 to 10. During this period a crop of spermatozoa leaves the tubule, their place is taken by a similar crop into which the young spermatozoa with spherical nuclei of the third layer have developed, the growing cells of the second layer produce by their division another generation of young spermatozoa, the small growing cells of the outer layer increase in size and become the large cells of the second layer, and a new crop of young growing cells makes its appearance in the outer layer. Thus, during a single cycle each of the layers described has moved forwards one stage, and the entire process of develop-

[1] 'Quart. Journ. Mic. Sci.'

ment from the spore-cell to the fully-formed spermatozoa would occupy four cycles, during which time four fresh generations of cells would have been produced ; and so the process goes on.

The first part of this process, i. e. the origin of the new crop of growing cells, which makes its appearance in the outer layer, is the most difficult to make out. These cells are first seen at the stage of fig. 9, and are produced by the karyokinetic division of larger cells, which are also found in the outer layer (this division by karyokinesis is represented in fig. 8, *a″*). The parent cells appear to be derived from the spore-cells by a process of division by budding, and not karyokinesis, but it is difficult to feel certain about this. The spore-cells apparently increase in size, and divide by a process of budding, for this seems to be the explanation of an appearance such as is represented in fig. 7, *a′*, as if a small outgrowth made its appearance from one part of the nucleus, increased until the two parts were about equal in size, and then separated off. Of the two cells which result from this division one divides by karyokinesis and produces growing cells, but the other remains in the resting condition as a spore-cell, destined to repeat the process in the following cycle, and thus perpetuate the production of spermatozoa in the tubule.

During the time in which a new crop of small-growing cells has been produced, the growing cells of the previous generation have been increasing in size, and now, since the new cells have appeared between them and the wall of the tubule, come to form the second layer. The manner in which this growth takes place will be understood if they are followed through the series represented by Pl. XXII,[1] figs. 1—10, *b, b′*, and fig. 1, *b″*. In fig. 1 these cells are all in the resting condition, and their nuclei present the ordinary appearance of resting nuclei, but very soon they begin to pass into the kinetic condition, the nuclear membrane disappearing and the chromatin becoming converted

[1] Figs. 3, 8, 9, and 10 are drawn on a somewhat smaller scale than the others.

into an irregular skein of filaments (fig. 2, *b'*). In this condition they increase in size without dividing, and gradually leave the wall of the tubule, until by the division of the spore-cells and formation of a new crop of cells between them and the basement membrane, they come to form the second row (fig. 8, *b'*). By the time the stage of fig. 1 is reached the nuclei of these cells have attained their full size, but the protoplasm continues to increase in amount. The nuclei are spherical and large (diam. 10 μ), and present the appearance represented in the figure (fig. 1, *b''*). A large granule—the accessory corpuscle—which stains darkly with chloride of gold, is embedded in the protoplasm near the nucleus (fig 12, *b''*). Even at the stage of fig. 1 a cell is occasionally seen containing two nuclei, one being placed internal to the other; apparently all the cells when their growth is completed divide into two, though the nuclei do not as yet show any further karyokinetic changes, so that at a later stage the cells are arranged in a double row.

Sometimes a nucleus may be seen apparently in the act of dividing, and cells containing two nuclei become more frequent (fig. 5, *b''*) ; and now the growing cells having reached their full development, divide by karyokinesis, the phenomena of which may be very well observed, and give to a tubule in this stage a very characteristic appearance (Pl. XXII, fig. 6, *b'''*). The astral and diastral forms can be readily recognised, and, viewed in profile, the achromatic filaments may also be seen. (Fig. 6 represents the appearances presented by these cells under a magnifying power of about 750 diameters.) In chloride of gold preparations the accessory corpuscle appears to become broken up during karyokinesis; perhaps it forms the accessory corpuscles of the young spermatozoa, and some small granules which stain slightly with hæmatoxylin and appear to be produced during karyokinesis may represent this process.

It appears that the cells which result from the karyokinetic division of a growing cell do not separate from one another entirely, but remain at first united in groups by a very small amount of the protoplasm of the mother cell. The outlines of the cells of which these groups are composed cannot be

made out from sections stained with hæmatoxylin, but in
chloride of gold preparations the cell outlines are rendered very
distinct (Pl. XXIII, fig. 14, *c*). The nuclei are spherical, of a
diameter of 5 μ, and are faintly stained by hæmatoxylin; they
possess a nuclear membrane, and a scanty network of chromatin.
Between the nucleus and protoplasm of the cell a clear space
makes its appearance, fitting like a cap over part of the nucleus,
and in the neighbourhood of the clear cap a small chromatic
granule is usually to be seen (Pl. XXII, figs. 8, 9, *c*).

Stained with chloride of gold, the young spermatozoa presents
an appearance such as is represented in fig. 14. There is a
small accessory corpuscle attached to the nucleus, which
subsequently becomes separated from it by a small vesicle
which seems to be derived from the accessory corpuscle (fig.
14, *c*).

A few minute fatty granules are seen dotted about in the
protoplasm of these cells, in osmic acid preparations, and the
accessory corpuscles are also to be seen (fig. 15). It is
difficult to make out clearly what is the origin of the clear
space which is seen in hæmatoxylin preparations between
part of the nucleus and the protoplasm of the cell; apparently
it corresponds to the small vesicle seen in chloride of gold pre-
parations, attaching the accessory corpuscle to the nucleus,
which may be increased in size by the action of alcohol on the
fresh cell.

The groups of cells are separated from one another for a
short time by bundles of spermatozoa, which extend between
them (fig. 9), but when the spermatozoa have left the sup-
porting cells, it becomes difficult to make out a separation into
groups, and there appears to be a layer of cells, three or four
deep, between the growing cells and the spermatozoa, for now
only the thin protoplasmic strands of the supporting cells
extend inwards between the groups, to the layer of granules in
which the heads of the spermatozoa are now embedded (Pl.
XXII, fig. 10). And now the stage of fig. 1 is again reached. In
this stage the young spermatozoa present a different appearance;
they are apparently free in the tubule, having been set at

liberty by the disintegration of the original groups. The nucleus now occupies the outer part of each cell, i. e. that directed towards the wall of the tubule, and the outer part of each nucleus is covered only by the clear cap. At the same time the chromatin leaves the substance of the nucleus and accumulates at the nuclear membrane, and chiefly at its outer part which becomes thickened, while the nuclear membrane in other parts seems to disappear, so that the nuclei appear to be breaking up. It is apparently such an appearance as this, represented by fig. 1, c, which has led V. Ebner and other observers who have investigated the spermatogenesis of the Rat, to the belief that these cells undergo liquefaction, and produce the liquid portion of the semen, or serve for the nutrition of the spermatozoa (vide the account of spermatogenesis given by Professor Schäfer in Quain's 'Anatomy,' vol. ii, ninth edition), and the granules of chromatic material which are found between these cells and the spermatozoa, have been attributed to the disintegration of nuclei. The accessory corpuscle leaves the nucleus at this time, and becomes embedded in the protoplasm at the inner part of the cell (fig. 12, c), where it remains inactive during the development of the spermatozoon, and is finally cast off with a residual portion of the protoplasm, when the process of development is nearly completed.

The young spermatozoa are free in the tubule for a very short time only, and they now begin to form groups in connection with the supporting cells. The remainder of their development takes place in these groups and occupies the fourth and last cycle.

The manner in which the connection between the young spermatozoa and the supporting cells is brought about is not very clear; apparently the young spermatozoa congregate round the processes of the supporting cells which extend towards the lumen of the tubule, and become embedded in the protoplasm, without of course any fusion between the substance of the young spermatozoa and of the supporting cell taking place ; but the appearances presented by the supporting

cells and young spermatozoa at this stage (fig. 1) are difficult to explain.

At this stage some supporting cells are seen which contain a more or less conical nucleus, situated in the outer layer upon the wall of the tubule, from which a protoplasmic strand extends inwards through the third layer of cells as far as the granules among which the heads of the spermatozoa are embedded; but the nuclei of other supporting cells appear to be pushing their way towards the lumen of the tubule; they are elongated in a radial direction, and may be seen between the second and third layers, and even in the middle of the third layer of cells, but farther inwards than this I have never seen them. In many cases the nuclei are seen to be connected to the outer layer by protoplasm, so that probably this is a migration of the nucleus and not of the entire cell (figs. 1 and 1, A). At the same time some of the young spermatozoa appear to move in the opposite direction towards the wall of the tubule, so as to occupy the position in the outer layer vacated by the supporting nucleus (fig. 1, A). These appearances at first appeared to me to justify the supposition that the supporting cells which have finished their work, having served for the support of the crop of spermatozoa which has just been discharged, are now in their turn being cast off into the lumen of the tubule, there to undergo disintegration, and that the cells which are passing outwards towards the wall of the tubule are destined to become the new supporting cells, retaining their connection with their brother cells, which develop into spermatozoa; so that according to this view the supporting cell and the group of spermatozoa which it supports would result from the division of a single cell.

But there are numerous objections to such a view as this:

1. The young spermatozoa at this stage are apparently free, and not connected together in groups.

2. It is very difficult to believe that, of a group of cells which are all exactly alike, and are produced by the karyokinetic division of a single cell, that which happens to be most ex-

ternal should become a supporting cell, while the others all develop into spermatozoa.

3. This improbability is rendered still more glaring by a comparison with the corresponding elements in the testes of the hedgehog and other animals, for in the hedgehog the nuclei of the supporting cells and of the young spermatozoa are remarkably dissimilar.

4. Nuclei of supporting cells cannot be seen in the lumen of the tubule, or in the semen from the vas deferens or epidydymis, and the nuclei which have migrated inwards show no signs of disintegration.

5. In the stage of development immediately succeeding this (*vide* fig. 2) nuclei of supporting cells are seen, which are quite as large as those in the present stage.

On all these grounds, then, it is impossible to adopt this view of the origin of the connection between the supporting cells and the spermatozoa. Probably the migration of the nuclei into the midst of the young spermatozoa has something to do with bringing about this connection, and having accomplished this the nuclei return to the outer layer.

The grouping of the young spermatozoa becomes more evident as soon as the preceding generation of spermatozoa with the seminal granules has left the tubule (this stage is represented by fig. 2). Each group contains about ten or twelve spermatozoa. At this time a curious appearance is presented by the supporting cells themselves; large globules, which stain black with osmic acid, are seen in the protoplasm near the nuclei; these globules are not simply fat-globules, since they stain very darkly with gentian violet, and in chloride of gold preparations become quite black from the great affinity which they have for the metal (*vide* fig. 13). In sections stained with hæmatoxylin vacuoles are seen in the protoplasm of the supporting cells, each of which contains a spherical body, which is slightly stained by the reagent; so that they would appear to consist of a mixture of fatty and albuminoid material. In many cases these bodies are found indenting the nucleus of the supporting cell, and sometimes look as though they were

protruded from the nucleus (*vide* Pl. XXII, fig. 2, *e* and fig. 11).

I was at first inclined to attribute the appearance of these bodies to a process of disintegration of the nuclei and protoplasm of the supporting cells taking place at this stage. If this were the case it would be very difficult to understand how these cells could be so quickly reproduced, for in the next stage of development (fig. 3) the nuclei of the supporting cells are fully developed, and present no signs either of growth or of degeneration, although a few fatty globules may still be seen in their protoplasm. Prof. Schäfer, however, pointed out to me that the appearance of these globules is probably due to an increased nutrition of the supporting cells taking place at this time, when they are about to enter upon a new phase of activity, and to serve for the support and probably also for the nutrition of a fresh crop of spermatozoa; and this appears to me to be the most probable explanation. Apparently the same supporting cell serves for the support of several successive crops of spermatozoa. I have not, however, been able to make out in what manner this reproduction takes place, and can say little about their life-history.

It is much easier to make out the history of the supporting cells in the Elasmobranch testis, which contains in a single transverse section every stage of development, from the embryonic condition to the fully-formed spermatozoa. The result of my own investigations on the testis of the dogfish is in agreement with the opinion of [1] Swaën and Masquelin, that while the spermatozoa are derived from primitive male ova, the supporting cells are descended from follicular cells, corresponding to the cells of the Graafian follicle in the ovary. It is probable that the same is the case in the mammal, but in order to make out the origin of the supporting cells in the mammal it would be necessary to trace them back to the embryonic condition.

The function of the supporting cells appears to be in great

[1] " Étude sur la spermatogenèse," par A. Swäen and H. Masquelin, 'Archives de Biologie,' tom. iv, fasc. 3, 1883.

measure mechanical; they serve the purpose of supporting and keeping in order the complex testicular epithelium, forming a sort of sustentacular framework like that of the Müllerian fibres of the retina. They serve for the support of, and also probably convey nutritive material to the young spermatozoa during their development, and when this is completed they expel them into the lumen of the tubule.

I must now return to the history of the development of the spermatozoa, at the point at which I left off to describe the supporting cells. We have at present reached the stage of fig. 2 in the fourth cycle.

A young spermatozoon at this stage is somewhat conical in shape, the rounded apex of the cell, which is directed outwards, being occupied by the nucleus.

The nucleus has become oval and projects from the cell protoplasm so that its outer hemisphere is covered only by the clear cap. As the development progresses the nucleus lengthens out, and projects more and more from the cell, until finally only its inner extremity remains embedded in the protoplasm. The projecting part of the nucleus is covered by the clear cap which increases with it, but when the hooklike form of the nucleus is established (fig. 6, *c'*) the clear cap is no longer to be seen.

As the nucleus increases in length it diminishes in thickness, and becomes more and more intensely stained by hæmatoxylin; there is no chromatic network, but the chromatin appears to be uniformly diffused throughout its substance. Before long the nucleus begins to curve (fig. 4) and the hooklike shape of the head of the spermatozoon is established by the time the stage of fig. 6 is reached.

The remainder of the process is occupied chiefly by the development of the body and tail of the spermatozoon, and can be studied much more satisfactorily from teased osmic acid preparations, from which the series of drawings (figs. 16—24) is taken. Growing spermatozoa at an early stage corresponding to fig. 14 are represented by fig. 22, *a*. They are small cells, elongated in a radial direction and contain a spherical nucleus

situated at about the centre of the cell; the accessory corpuscle is to be seen in these cells attached to the nucleus by a minute vesicle, and another small refracting granule is attached to the nuclear membrane, at the point from which the development of the body of the spermatozoon is about to begin. There are also a few minute fatty granules dotted about in the protoplasm (fig. 15, c). The manner in which these cells develop into spermatozoa is represented by fig. 22.

The nuclear membrane over the outer hemisphere of the nucleus becomes slightly thickened, and at the opposite pole of the nucleus, where the small granule is attached, a fine filament makes its appearance in the protoplasm, and extends from the nucleus to the surface of the cell, where it is prolonged by a delicate protoplasmic filament, the rudiment of the tail of the spermatozoon. At this time the[1] accessory corpuscle breaks away from the nucleus and becomes embedded in the protoplasm at the inner part of the cell, where it remains inactive during the remainder of the process, to be finally cast off with the protoplasmic residue when the development is nearly completed. The protoplasm of the cell now becomes collected entirely at the inner part of the nucleus, leaving the outer hemisphere, upon which is the thickened membrane, covered only by the clear cap (22, d).

This corresponds to the stage of development represented by figs. 1 and 12, at which the young spermatozoa appear to be free in the tubule. The nuclei of the cells are now commencing to elongate in the radial direction and to take on the oval form, and a small prominence becomes visible at the outer pole of the nucleus at the centre of the thickened membrane, the " bouton terminal " of Renson (fig. 22, e). It is at this stage that the grouping in connection with the supporting cells is first seen. Fig. 16 represents a group of young spermatozoa embedded in the protoplasm of a supporting cell, but the nucleus of the body cannot be seen, and the protoplasm appears to be

[1] I have not been able to make out what the origin of the accessory corpuscle is. Perhaps it is derived from the nucleus of the growing cell.

broken off short. This, however, is readily explained, when it is considered that the tubules were broken up with needles, by which process the inner part of the supporting cell containing the young spermatozoa becomes broken off from the base which contains the nucleus, and is found to remain adherent to the basement membrane.

The manner in which the oval nucleus becomes transformed into the head of the spermatozoon will be understood from fig. 22. The nucleus increases in length and projects more and more from the cell, and the thickened part of the nuclear membrane progresses so as to cover the whole of the projecting portion. Soon the nucleus begins to curve, the curvature first appearing near the apex, presumably owing to an increased growth of one side of the thickened membrane. As development goes on the curvature increases, and the denser portion involves more and more of the substance of the nucleus.

The thickening of the nuclear membrane is apparently due to a condensation of the nuclear substance and its transformation into that of the head of the spermatozoon, which, beginning at the surface and at the outer pole of the nucleus, progresses until the whole nucleus is converted into the spermatozoon head.

During this time the cilium which springs from the inner extremity of the cell has reached a considerable length, but very little progress has been made with the development of the middle piece of the spermatozoon, although the cell protoplasm has elongated to some extent.

The young spermatozoon is now (fig. 22, *l*) pyriform in shape, the base of the hooklike nucleus being inserted into the narrow end of the cell, a long cilium springs from the broad end, and connecting the nucleus to the cilium is a delicate filament which can only be seen with some difficulty (*vide* figs. 17 and 6, *c′*). The remainder of the process is occupied chiefly by the development of the middle piece of the spermatozoon (figs. 18—20). The cell protoplasm rapidly elongates and assumes a club-shaped form, since the upper extremity which

contains the accessory corpuscle and some fatty granules remains bulged (figs. 18 and 23). The filament which joins the nucleus to the cilium is more plainly seen in the narrow part of the cell. This lengthening of the spermatozoon is accompanied by a corresponding movement of the heads downwards along the protoplasm of the supporting cell, until when the spermatozoa have attained their full length their heads reach the outer layer, in the neighbourhood of the nucleus, where they remain until the spermatozoa are cast off into the lumen. A group at this stage is represented by figure 19. The middle piece of the spermatozoon is now clearly visible passing through the protoplasm, which is collected chiefly at its upper end near the junction with the tail. The middle piece is formed out of the protoplasm of the cell, but not, as might be supposed, from the whole of the protoplasm, for a residual portion separates off from the spermatozoon, when its development is nearly completed. This residual part of the protoplasm, which contains the accessory corpuscle and one or two clusters of small fatty granules, gradually accumulates in the form of a globule, which separates from the body of the spermatozoon. At first the globule remains attached to the upper part of the body by a short pedicle (a group of spermatozoa at this stage is represented by fig. 20, and columns of spermatozoa in situ with the globules attached in fig. 15), but before long it breaks away entirely from the spermatozoon.

In sections stained with hæmatoxylin small chromatic granules make their appearance in the columns of spermatozoa (fig. 9, x), and when the spermatozoa have passed into the lumen these granules, each of which is contained in a small amount of protoplasmic material, are found detached from the columns, and occupying the interval between the heads of the spermatozoa and the cells of the third layer. These bodies have been previously described as the seminal granules; they are, in fact, the globules which, as we have just seen, separate from the spermatozoa at a late stage of their development, and the chromatic granules seem to be formed in part by the clusters of fatty granules seen in osmic acid preparations (figs. 10 and

1, x).   This separation of globules is of considerable interest
from a biological standpoint, for it apparently corresponds to
the separation of polar globules from the ovum, and may re-
represent the elimination of the female element from the
spermatozoa.   Apparently the ova and spermatozoa are derived
from similar embryonic cells—the primitive ova, which are
hermaphrodite.   In a cell which is destined to develop into
spermatozoa the male element predominates, and increases
until, by the separation of the globules, the spermatozoa
become wholly male, while in the cell which is going to become
an ovum the female element predominates, and by the separa-
tion of the polar globules the cell becomes unisexed and ready
to be fertilised by the addition of a new male element.

In invertebrate animals the separation of the female element
would seem to take place at an earlier period during the pro-
duction of the spermatozoa, and not, as in the present in-
stance, during the development; and this is probably the
meaning of the blastophoral body, as described by Blomfield
in the case of the earthworm.[1]   In this animal the young
spermatozoa undergo their development in groups—" the sperm
polyblasts."   The " sperm polyblast" is a mulberry-like mass,
which results from the repeated division in geometrical pro-
gression of a single cell—" the spermatospore," or male ovum;
during each division a certain amount of the protoplasm of the
mother cell remains behind, connecting together the daughter
cells; this residual protoplasm accumulates at the centre of the
group of cells, so that when the process is completed the sperm
polyblast is composed of a central protoplasmic body—the
" sperm blastophore," which is covered all over by " sperma-
toblasts," or young spermatozoa, which remain planted on the
blastophore until their development is completed.

This interpretation of the separation of the globules, and
the comparison with the blastophore of the earthworm, was
suggested to me by Professor Lankester,   The blastophore of
the earthworm, though it has much the same function as the

[1] " The Development of the Spermatozoa," part i, " Lumbricus," by J.
E. Blomfield, B.A., 'Quart. Journ. Micr. Sci.,' Jan., 1880.

vertebrate supporting cell, has a different morphological significance, the supporting cells being probably derived from follicular cells which appear not to be represented in the invertebrate testis.

As soon as the separation of the globules has taken place, or even before this, the spermatozoa begin to travel bodily towards the lumen of the tube. This movement is apparently produced by the supporting cells, which convey the spermatozoa inwards to the lumen of the tube, where they finally become detached. A supporting cell which is thus casting off its group of spermatozoa, is represented by fig. 21. For a short time the head of the spermatozoon remains embedded in a protoplasmic envelope, perhaps derived from the supporting cell, and a small granule of protoplasmic material, darkly stained in gold preparations, remains for some time at the junction of the head with the middle piece (fig. 24, *b*, *c*), but eventually disappears.

A mature spermatozoon, examined fresh, or mounted in glycerine after osmic acid, shows no trace of a division into body and tail, appearing to be composed of two parts only, the head and the long tapering body; but by treatment with chloride of gold, as before described, the division into middle piece and tail is rendered very conspicuous.

Fig. 25 represents a spermatozoon from the epidymis, which is mounted in glycerine after having been treated with chloride of gold. The middle piece is somewhat swollen and stained by the reduction of the gold, the staining being chiefly concentrated in a fine spiral fibre, which winds closely round this portion. It has a length of about ·07 mm., and presents a striking contrast to the tail, which is absolutely unstained; the length of the tail is about ·08 mm. The spiral filament, as seen in sections mounted in balsam, has been already described (fig. 12, *d*). I have not been able to make out the manner of its development. It is first seen when the spermatozoa have reached their full length, before they have begun to travel to the lumen of the tubule (fig. 14).

I will now give a brief account of some of the different
views upon mammalian spermatogenesis which have been put
forward during the last fifteen years, describing chiefly those
which are of most interest for the purpose of comparison.

For this I am to a considerable extent indebted to the
digest of the literature upon the subject which is given by
Renson in the 'Archives de Biologie' for 1882, in his paper
upon " Mammalian Spermatogenesis."

Von Ebner, in 1871, gave an account of spermatogenesis,
taken from a study of the testis of the Rat by means of sec-
tions, which has received a good deal of support.

The supporting cells are described under the name of sperma-
toblasts, and are considered to be the parent cells of the
spermatozoa, which are formed endogenously from the proto-
plasm of the spermatoblasts. Von Ebner describes an external
layer, resting upon the wall of the tubule, composed of two
kinds of cells, which differ from one another in the appearance
of their nuclei, some of them having large nuclei which con-
tain a spherical nucleolus, and others containing small granular
nuclei. The cells with the large nuclei are the " spermato-
blasts." The protoplasm of each spermatoblast joins that of
its neighbour on each side, to form a sort of protoplasmic
network upon the wall of the tubule, in the interstices of which
are contained the small cells with granular nuclei. On the
inner side the spermatoblast gives off a protoplasmic process,
which extends radially towards the lumen of the tubule. The
inner extremity of this process enlarges and splits up into
digitations, and at the base of each digitation a nucleus de-
velops, being formed out of the protoplasm of the mother cell;
then the nucleus elongates and becomes the head of a sperma-
tozoon, a filament grows out from the extremity of each digi-
tation and forms the tail, while the protoplasmic digitation
itself is converted into the middle piece. During their de-

velopment the heads of the spermatozoa travel downwards towards the base of the cell, so that they reach the outer layer; on the completion of the process they travel back again, and finally are thrown off into the lumen. The "round cells," which occupy the spaces between the columns of spermatozoa, according to von Ebner, serve only for the production of the liquid portion of the semen.

It is obvious that this account is due to an erroneous interpretation of the appearance of columns of spermatozoa, which is so conspicuous a feature in the testis of the rat; there cannot be the least doubt that it is from the "round cells" that the spermatozoa are derived, and that they are not produced endogenously in the protoplasm of the supporting cells.

Merkel, in 1871, gives a very different account of the process to that of von Ebner. He considers that the spermatozoa are derived from the small round cells which, according to von Ebnor, undergo liquefaction; these become embedded in cavities which are hollowed out in the protoplasm of the supporting cells, thus producing the spermatoblasts of von Ebner, and in these supporting cells undergo development into spermatozoa.

Sertoli, in 1874, gives a much fuller account of the process. He describes fixed, or supporting, and mobile cells, which he divides into three classes. 1. "Germinative" cells, which are small, and situated in the outer layer between the bases of the supporting cells. 2. "Seminiferous" cells, which are larger, and form the second layer, and correspond to the germinative cells of the preceding generation, which have increased in size, and become pushed inwards by the formation of a new layer of germinative cells, between them and the wall of the tubule. 3. "Nematoblasts," which are small cells produced by the division of the preceding generation of seminiferous cells, and destined to develop into spermatozoa. Sertoli gives no account of the mode of production of the germinative cells.

Lavalette St. George, who has published numerous papers

upon spermatogenesis, takes a different view. He considers that the connection between the spermatozoa and the supporting cells is primary, both being derived from the division of a single cell. These cells are situated in the outer layer between the germinative cells of Sertoli, which, according to this observer, are follicular, and take no share in the production of spermatozoa. They divide in a radial direction into two cells, which do not entirely separate from one another. The external of the two remains in the resting condition attached to the wall of the tubule, and is called the spermatogonium, while the other cell increases in size and its nucleus repeatedly divides, so that a multinucleated mass is produced—the "spermato-gemme;" this becomes segmented, and each segment develops into a spermatozoon. The cell at the base, or spermatogonium, retains its connection with the spermatozoa until their development is completed.

Somewhat similar accounts have been given in 1880 by Meyer, in the 'Memoirs of the St. Petersburgh Academie,' and by Brissand in the 'Archives de Physiologie.'

Helmann in 1879, and W. Krause in 1881, agree with Lavalette St. George in considering the supporting cell and the spermatozoa to be derived from the same parent cell, but agree with Sertoli that the germinative cells of the outer layer are the progenitors of the spermatozoa. They consider that one of the nuclei of the spermatogemme migrates towards the wall of the tubule, passing between the cells of the second layer to become embedded in the outer layer upon the basement membrane, and that this nucleus, retaining its connection with the spermatogemme and increasing in size, becomes the supporting nucleus, while the spermatogemme develops into a group of spermatozoa; so that, according to this view, there are no follicular cells in the tubules. I myself held for some time such an opinion as this upon the relation between the spermatozoa and the supporting cell, and have already explained at some length why I felt obliged to give it up.

Klein, in the 'Atlas of Histology,' in 1881, gives an account of the development of spermatozoa in the dog and some other

mammals. He describes the development of spermatozoa from small cells resulting from the division of the inner seminal cells; these daughter-cells are at first free in the tubule, but gradually form fan-shaped groups, which sink between the inner seminal cell towards the wall of the tubule.

Klein has not observed the existence of supporting cells, to which the groups of spermatozoa are attached, and offers no explanation of this grouping.

Schäfer, in the ninth edition of Quain's 'Anatomy,' in 1882, gives a short account of the testis of the Rat; he attributes to the small cells of the third layer a nutritive function, and considers that some of the proliferating cells (the large cells of the second layer) give rise by their division to groups of sper-matozoa, while others form the small cells, which ultimately break down and liquefy.

Renson, in the 'Archives de Biologie,' 1882, gives a descrip-tion of mammalian spermatogenesis, taken chiefly from a study of the process in the Rat, which agrees very closely in most points with the result of my own investigations.

Renson traces the origin of the spermatozoa to the small round cells of the outer layer, which he calls after Sertoli, "germinative" cells, and which make their appearance sud-denly in the outer layer upon the basement membrane, but he has not been able to discover in what manner this new layer of cells is produced, and the production of spermatozoa perpetuated.

The germinative cells increase in size, and become in the next cycle the "seminiferous" cells which form the second layer. The seminiferous cells divide by karyokynesis into groups of daughter cells, which he calls "cysts." The "cysts" disintegrate, and their component cells, the "nematoblasts," are set free. Soon they contract a connection with the supporting cells, in which they become embedded in groups. Finally, when this development is completed, they are thrown off by the supporting cells into the interior of the tubule. Renson also describes the appearances presented by the nematoblasts during their development, as studied by teased preparations

and his account of the process agrees very closely with that which I have given, but he does not describe the separation of the globules of residual protoplasmic material which takes place when the development is nearly completed. He describes the accessory corpuscles, both in the seminiferous cells and in the nematoblasts, and suggests that they may represent the polar globules of the ovum.

Swaën and Masquelin, in the 'Archives de Biologie' for 1883, give the results of their investigations upon spermatogenesis in the Selachians, the Salamander, and the Mammal. Their account of the process in the mammal was taken from a study of the testis of the Bull, and agrees in many particulars with that of Renson. These observers give an acccount of the manner in which the continual production of succeeding generations of spermatozoa is kept up in the tubule, which presents some resemblance in principle to the view of the process which I have taken, though differing from it in detail. They call the small cells of the outer layer—the germinative cells of Sertoli and Renson—while they are in the resting condition "inactive male ovules." These cells passing into the kinetic condition become the active male ovules, and gradually leave the wall of the tubule. Before long each cell divides into two by karyokinesis in a radial direction; the external of the two cells becomes embedded in the outer layer, and passing into the resting condition, becomes an inactive male ovule, which repeats the process in the succeeding cycle. The other cell, which is internal, increases in size, and finally divides by karyokinesis into a group of cells, the "spermatogemme."

The cells of which the spermatogemme is composed are called spermatocytes, and afterwards, when they have obviously begun the process of development into spermatozoa, receive the name of nematoblasts. The nematoblasts become attached to the supporting cell without being first free in the tubule, for the inner extremity of a supporting cell which has discharged its spermatozoa fuses with the intercellular material of the spermatogemme.

The account which I have given of the origin of the growing

cells of the outer layer from spore cells, which divide in the first instance by a process of budding, and the subsequent division of one of the resulting cells by karyokinesis, has not been confirmed by any previous investigations. It is possible that I may have been misled by the appearance of division by budding which is occasionally to be seen in these cells in the outer layer, and is represented in fig. 7, *a'*, consequently I have been much interested in finding a similar method of division by budding of the nucleus described by Arthur Bolles Lee, in a recent [1] paper on spermatogenesis in the Appendicularia. This observer suggests a theory to explain the occurrence of the two methods of cell division, which is an ingenious one, and certainly fits in very well with the account of the process which I have given above.

He suggests that the complex method of division by karyokinesis is intended to serve for the accurate division of the constituents of the nucleus between the resulting cells, so that the daughter cells, possessing in an equal degree the properties of the parent nucleus, exactly resemble one another. On the other hand the method of division by budding consists of an elimination of one part of the nucleus from the remainder, so that the resulting cells will not exactly resemble one another.

I have described above the spore cell as dividing by budding, and of one of the resulting cells remaining as a spore cell while the other divides by karyokinesis, so that the result of the division by budding is to produce two dissimilar cells. On the other hand, the cell which divides by karyokinesis produces the growing cells which are all precisely alike, and these later on dividing again by karyokinesis give origin to the young spermatozoa which again are all alike.[2]

[1] " Recherches sur l'ovogenèse et spermatogenèse chez les Appendicularia," par Arthur Bolles Lee, ' Recueil Zoologique Suisse,' vol. i, No. 4.

[2] It might be supposed that the mode of separation of the polar globule from the nucleus of the ovum goes against this theory ; but it appears that, according to van Beneden, this is not a true process of cell division by karyokinesis (*vide* a paper on " E. van Beneden's Researches on the Maturation and Fecundation of the Ovum," by J. T. Cunningham, ' Quart. Journ. Micr.

EXPLANATION OF PLATES XXII & XXIII,

Illustrating Mr. Herbert H. Brown's Paper " On Spermatogenesis in the Rat."

PLATE XXII

Represents sections of tubules from the testis of the Rat, stained with hæmatoxylin.

Most of the figures in both Plates are drawn under a magnifying power of 750 diameters, but Figs. 3, 8, 9, 10, 14, and 15 on a slightly smaller scale.

Figs. 1—10 show consecutive stages in the production of spermatozoa. a. Spore-cell. a'. Ditto, dividing by fission. a''. Cells dividing by karyokinesis to produce the young growing cells. b. Young growing cell in resting condition. b'. Ditto, in kinetic condition. b''. Growing cell at a later stage (in the second row). c. Young spermatozoa with spherical nuclei. c'. Young spermatozoa undergoing development. d. Adult spermatozoa. e. Supporting cells. x. Seminal granules.

Fig. 11.—Nuclei of supporting cells, showing the large fatty-albuminoid globules (stage corresponding to Fig. 2).

PLATE XXIII.

Figs. 12 — 14. — Sections prepared with chloride of gold (lettered as Figs. 1—10).

Fig. 12. Corresponds to Fig. 1.

Fig. 13. Ditto to Fig. 5.

Fig. 14. Ditto to Fig. 9.

Fig. 15.—Section stained with osmic acid. Stage corresponding to Figs. 14 and 9.

Figs. 16—20.—Groups of developing spermatozoa from osmic acid preparations, mounted in glycerine.

Fig. 16. A group of young spermatozoa with oval nuclei.

Fig. 17. Group of young spermatozoa at stage of Fig. 6.

Fig. 18. Young spermatozoa slightly more developed, showing elongation of the protoplasm.

Sci.,' January, 1885, p. 107). In this paper the theory as to the separation of the female element from the spermatozoon and the male element from the ovum is also brought forward.

Fig. 19. Group of young spermatozoa, stage of Fig. 8. (The middle piece of the spermatozoa has now reached its full length.)

Fig. 20. A group of spermatozoa, showing the separation of the seminal granules.

Fig. 21.—A supporting cell casting off its spermatozoa.

Figs. 22—24.—Osmic acid preparation. Separate spermatozoa.

Fig. 22 (*a—l*). A series showing the development of young spermatozoa, detached from the groups. The gradual transformation of the nucleus into the spermatozoon head is especially shown.

Fig. 23. A spermatozoon at the stage of Fig. 18.

Fig. 24. Spermatozoa almost fully developed. *a*. The residual globule is still attached. *b*. This is thrown off, but the head of the spermatozoon is not yet free. *c*. There is only a small granule remaining at the junction of the head and middle piece.

Fig. 25.—A spermatozoon from the epidydymis of the Rat, stained with gold, showing the spiral filament.

Fig. 1   Fig. 1a

Fig. 11

Fig. 9   Fig. 10
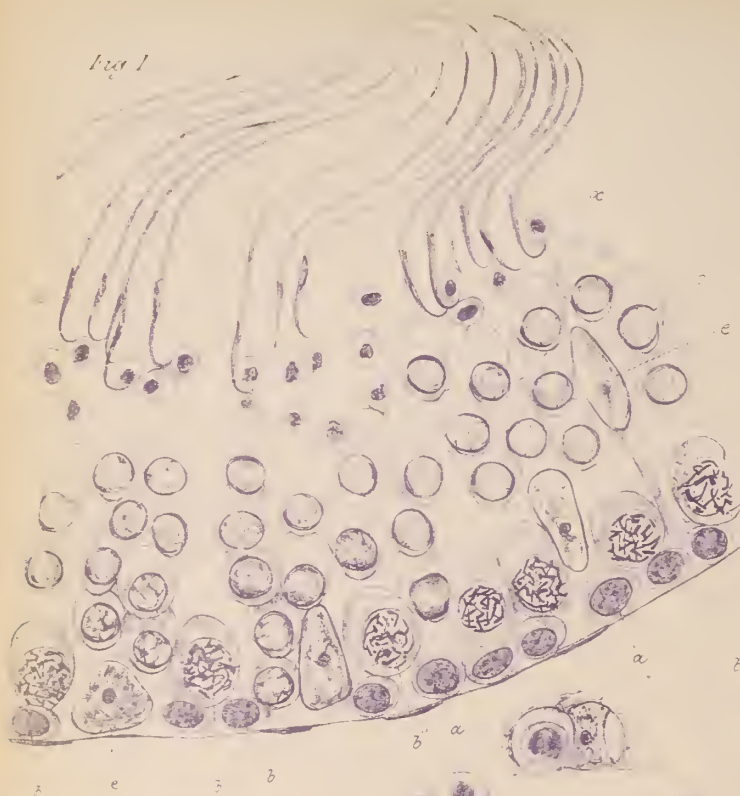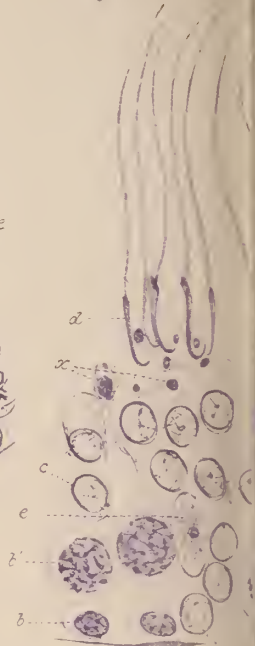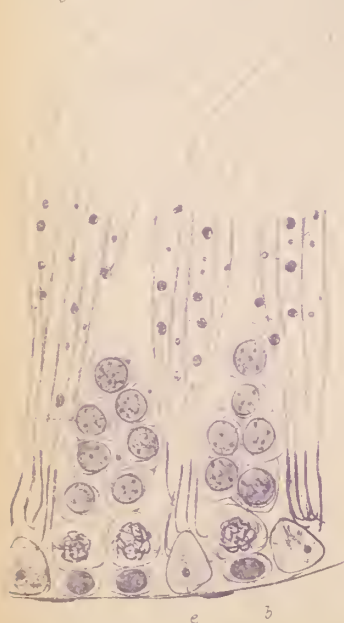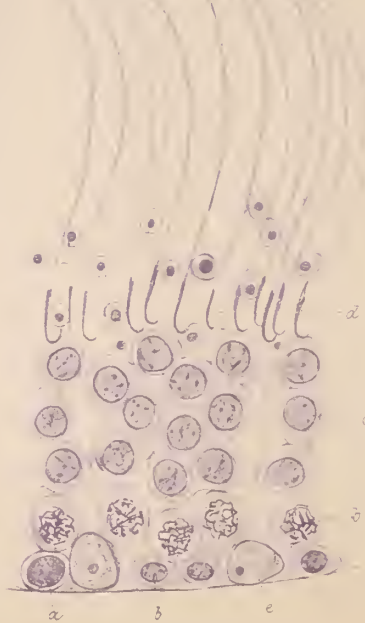
Fig. 6
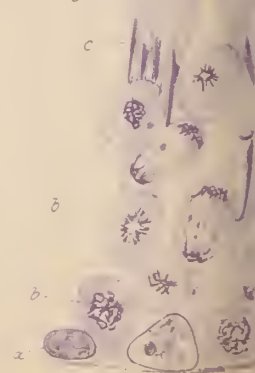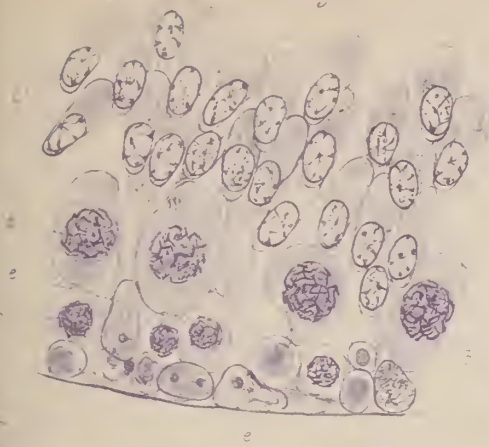
Fig. 2

Fig. 3.
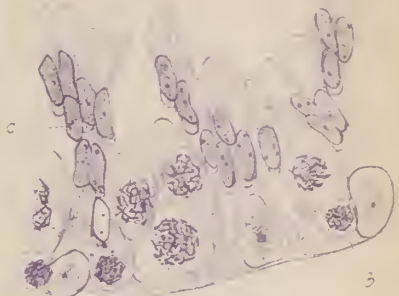
Fig. 5.

Fig. 4.

Fig. 7
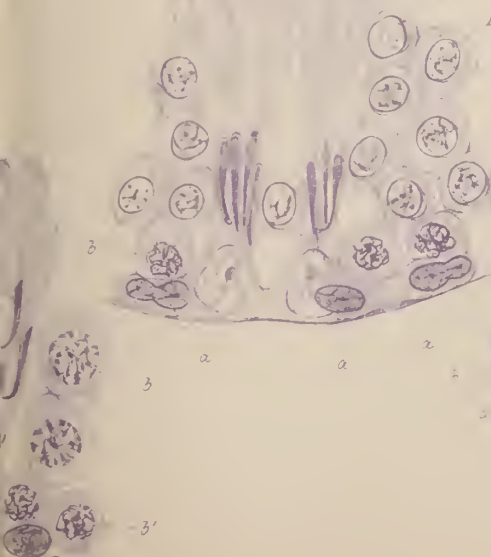
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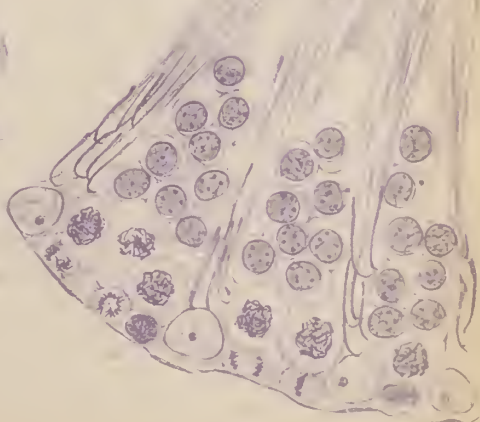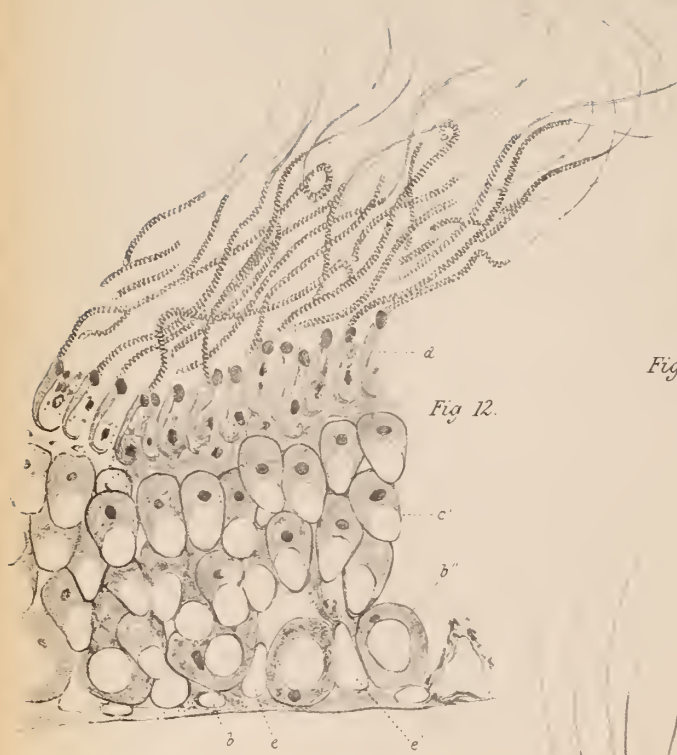
Fig 12.
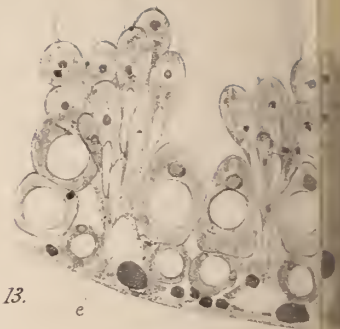
Fig. 13.

Fig 17.

Fig. 18.

Fig. 21.

Fig. 19.

Fig 16.
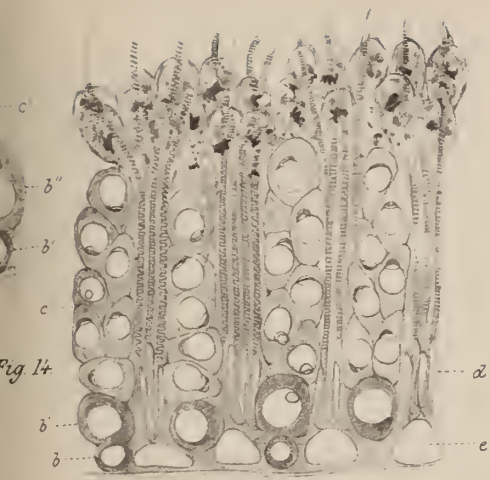
Fig. 14.

Fig. 15.
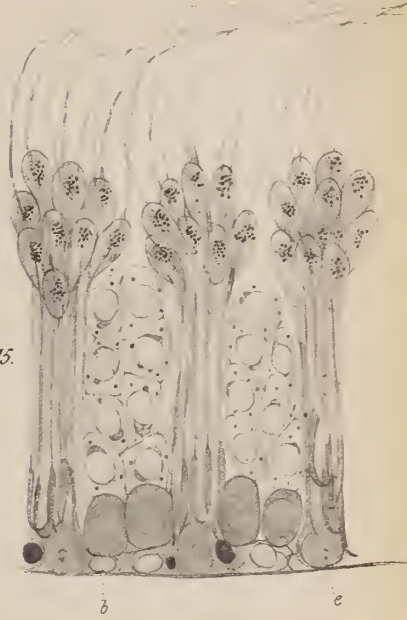
Fig. 20.
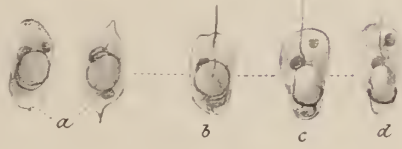
Fig. 22.

Fig. 23.
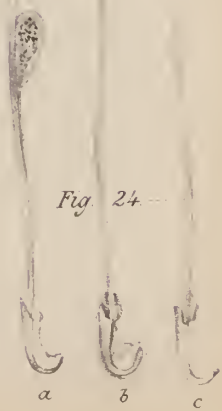
Fig. 24.

Fig. 25.

A Simplified View of the Histology of the Striped Muscle-Fibre.

By

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With Plate XXIV.

Introduction.

Everyone who has considered the subject must admit the essential identity from a physiological point of view of all those tissues which possess in a special degree contractility. The contraction of a white blood-corpuscle or amœba is essentially the same phenomenon as the contraction of an involuntary fibre-cell or a striped muscle-fibre.

When we consider these three contractile tissues from a histological point of view we are struck by an apparently essential difference in character between the striped muscle-fibre and the elements of the other two contractile tissues, and indeed cells generally. The voluntary muscle-fibre is morphologically a cell like the muscle-fibre cell and the amœboid corpuscle. Yet it differs from the latter and from all other cells in showing a characteristic transverse striation.

According to Klein,[1] the protoplasm of the simpler contractile tissues, (1) the amœboid cell, (2) the ciliated cell, and (3) the involuntary fibre-cell, agrees, inasmuch as it consists of two parts—a matrix and an arrangement of fine fibrils, the intracellular network. The actual arrangement of

[1] 'Klein, 'Atlas of Histology,' diagrams 1 and 4, and fig. 2, pl. xv.

the fibrils differs somewhat in the three cases. In the white blood-corpuscle they are arranged into a network or meshwork with polygonal meshes. In the ciliated cell they also form a network which seems to be in peculiar relation with the cilia. In the ciliated cell of the Mollusc, according to Engelmann,[1] the fibrils are arranged in a longitudinal manner as fine varicose filaments running the whole length of the cell, and in connection with the bases of the cilia. In the protoplasm of the involuntary fibre-cell the fibrils are arranged in a central or axial bundle, anastomosing at the poles of the nucleus with the intranuclear network.

Observations on which I have been engaged for some time past, and which have been partly worked out in the Physiological Laboratory of Owens College, lead me to the belief that the striated muscular fibre really agrees fundamentally as regards histological structure with the other contractile tissue elements, in containing an intracellular network, differing from them merely in the greater amount of differentiation, and more regular arrangement of the network.

I believe, further, that the various conflicting descriptions given by different observers, and those points on which competent histologists differ more materially, can be explained and brought into harmony with one another by this view.

I have observed this network in the fibres of Dytiscus, the Bee, Crayfish, Lobster, Frog, and Rat prepared by a somewhat special method of gold staining, the network being the only part of the fibre stained by the gold.

It may be specially stained also by treating the fibre with acetic acid and subsequently staining with hæmatoxylin.

It may be demonstrated, though not so completely, in the living fibre, and in acetic and osmic acid preparations. I have submitted my drawings and preparations to the examination of Prof. A. Gamgee and Prof. A. Milnes-Marshall.

[1] Engelmann, 'Pflüger's Archiv,' xxiii, 1880, and 'Quain's Anat.,' 9th edition, vol. ii, fig. 240.

Demonstration of an Intracellular Network in the striped Muscle-Fibre.

I. The Muscle-Fibre prepared with Gold Chloride.

(a) Dytiscus marginalis.

Method of Gold Staining.—Decapitate a Dytiscus, open the thorax, remove a portion of a leg muscle, and place in 1 per cent. acetic acid for five to fifteen seconds, then into gold chloride solution 1 per cent. for forty-five minutes, and leave in formic acid 25 per cent. for forty-eight hours in the dark. Tease and mount in glycerine.

If now examined with a magnifying power of about 700 diam. the appearances seen in figs. 1, 2, 3, 12, 13, and 14 will be seen in certain of the fibres. The method of preparation has a great tendency to soften the fibre, so that it becomes much expanded on compression by the cover-slip; it also has a great tendency to split the fibre into transverse discs.

Fig. 1 represents a fibre which has retained its natural size and form. Narrow transverse bands of granular substance, deeply stained with the reduced gold, are seen crossing the fibre separated by wider bands of lighter substance. These deeply stained granular bands correspond in position to Krause's "membrane." The usual separation into light and dim discs of about equal thickness is lost by this method of preparation. Traversing the wider unstained discs, and giving the fibre the appearance of longitudinal striation, are seen fine longitudinal lines.

In fig. 2 is seen a portion of a fibre which has been more flattened out by pressure. In it the deeply stained, narrow granular band is seen to consist of a transverse row of dots. The longitudinal lines are seen to represent fine rod-like bodies traversing the position usually occupied by the dim stripe, and being continued into the dots at either end. In some fibres a minute thickening of the rod is apparent midway in the position of the so-called " Hensen's disc" (in the middle of the dim stripe).

This method, as was before stated, has a tendency to split
the fibre into transverse discs. These isolated discs are
found in many parts of the preparation; they present the
appearances seen in figs. 4 and 5. They are seen plainly in
all cases to consist of two parts—(1) a network of fine lines
highly refracting, stained by the gold, and having thickenings
at the nodes; and (2) an unstained substance lying in the
interstices of the network.

The appearance of this network differs somewhat with the
degree of compression of the discs. When much compressed
the network appears more open, and the nodal dots less
marked. Towards the outside of the fibre the meshes appear
more oblong, the network extending mostly in a radial direc-
tion. This network evidently corresponds when it is in its
transverse position in the fibre with the deeply-stained, beaded
disc occupying the position of Krause's " membrane." This
is shown in certain fibres in which the discs are not seen
perfectly edgeways but in perspective (fig. 6). The beaded
disc at each membrane of Krause is here seen to consist of
a transverse or horizontal network, united to the discs above
and below by fine thread-like lines. This method of
gold staining, then, brings out a network arranged in a
manner represented diagrammatically in diagrams 1, 2, 3,
and 4.

This network differs chemically from the rest of the fibre,
inasmuch as it resists to a larger extent the action of acetic
acid, and possesses in a greater degree the power of reducing
gold.

It will be shown later, by other methods of preparation, that
this network differs again from the matrix in its physical pro-
perties. The network is isotropous and highly refractile. The
refractive power is somewhat altered by gold staining, but
certain optical effects are still produced by the refractive action
of the network upon light. These optical effects can be more
definitely seen in isolated portions of the network than in
the whole fibre.

Optical Effects produced by the Network.

Fig. 12 represents a small piece of the network isolated from the rest of the fibre, consisting of nine or ten rows of dots and the connecting longitudinal bars.   There is a single layer only of network and dots.   This isolated piece seems to be a portion of sarcolemma stripped off the fibre, along with the portion of network immediately below the sarcolemma, and attached to it by each transverse network.

When exactly focussed (fig. 14, L) each dot appears as a dark granule surrounded by a bright halo.   The blending of these haloes causes a crenated bright transverse band.   The effect of alternating light and dim bands is thus obtained, the bright band being crossed transversely by a row of dots, the dim band longitudinally by a series of fine lines.

On altering the focus (raising ·0025 millimetre, about), the refractive effects are to a certain extent transposed (fig. 14, U). The dots now appear bright, surrounded by a dark border. By coalescence the appearance of a narrow bright disc is produced, separated from the dim disc at each side by a dark crenated line.

Similar refractive effects and transposition on focussing are seen in the discs isolated by transverse splitting of the fibre.

Transposition of the Bands.

The effect known as "transposition" of the bands has been noticed by many observers.   On raising the objective what was previously the bright band appears now darker than the dim band.

This so-called transposition is seen in fibres prepared by the gold method, better in fibres prepared with osmic acid; diag. 6, U represents a fibre at the upper focus.   The light band in the position of Krause's membrane appears very bright, and is bordered by a dark line at the junction of the light and dim bands.   On focussing about ·0025 mm. lower down (with Zeiss D obj.) the appearance seen in L is obtained.   The darkest

part of the fibre is now in the centre of what was the bright band, that is, in the position of Krause's membrane. Bordering on this dark band, and separating it from the dim band, is a bright zone. The dim band remains much the same as before, though by contrast with the now dark Krause's membrane it may appear lighter.

The bright haloes round the nodal dots of the network may be compared with the similar effects observed whenever any highly refractile particle, such as a micrococcus or minute oil-globule, is observed in a medium of lower refracting index.

In the oil-globule suspended in water similar and very definite transposition effects are seen on altering the focus. If focussed low it appears as a dark spot surrounded by a bright halo or border (L, diag. 7). On raising the objective (about ·0025 mm., Zeis D) the oil-globule appears bright, surrounded by a dark border.

The effect produced when a row of oil-globules are seen side by side is at the lower focus (L), a bright band (formed by the coalesced haloes) with a series of dark dots traversing it. At the upper focus (U) a narrower bright band, bordered by dark edges. The beads at the nodes of the transverse network may be looked upon as refracting and reflecting the light, in the same way as an oil-globule in water, and as causing the so-called "transposition" of the bands seen on altering the focus.

(*b*) Bee.—Insect muscle may be very conveniently obtained from the thorax or leg of the bumble bee.

Prepared with acetic acid and gold chloride, by the method already described, it shows a network identical with that described in Dytiscus.

In order to obtain muscle in as uncontracted a condition as possible, gold preparations were made from the leg muscles of a bee, rendered insensible and immovable by chloroform vapour, in which presumably there was complete relaxation of the muscle-fibre. These preparations, however, could not be distinguished from those prepared without chloroform.

As the fibres are rendered soft by the method of preparation their size and the size of their elements varies with the pressure of the coverslip; hence measurements are of little or no value.

Identity of Network with Schäfer's Muscle-rods.

We cannot but be struck by the resemblance of the appearances brought out by gold staining with those described by Schäfer[1] in the living fibre as muscle-rods. The two views differ, however, on two points : (1) Schäfer describes in a transverse section of the fibre a bright ground substance with a number of minute specks or dots; no appearance of a network. (2) He considers that there is typically a double transverse row of dots in the middle of each bright stripe.

Concerning the appearance on transverse section we must not forget that Schäfer's conclusions were drawn from the living fibre in optical transverse section. Probably he saw all that it is possible to see of the transverse network in the living fibre, namely, the thickenings or dots at the nodal points, the fine network, seen so plainly in a transverse view when stained with gold, not being visible in the fresh fibre examined in this way.

Is there a single or a double row of dots in the middle of the bright stripe ? In the fresh fibre sometimes a single sometimes a double row of dots is seen, the two appearances often alternating with a higher or a lower focus. The same variation is seen in alcohol and some other preparations.

In the gold preparations, when the fine granular disc or transverse network is seen perfectly edgeways and in focus, it appears invariably made up of a single transverse line of dots.

When the transverse network is not seen perfectly edgeways, through not lying in a plane quite at right angles to the longitudinal axis, but slightly obliquely or in perspective, it

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[1] 'Phil. Trans.,' xii, 1873, E. A. Schäfer " On the Minute Structure of the Leg Muscles of the Water-beetle."

may appear as a double row of dots or as a granular or dotted band crossing the disc transversely.

In a perspective view of the fibre (figs. 3, 6, and 17), not only the dots (nodal points of the network) at the near side of the fibre are seen, but at the same time those deeper down or at the far side. Hence the appearance of two or more rows of dots crossing the fibre. When by raising the focus the nearer edge of one of these obliquely-arranged discs is alone focussed it is seen to consist of a single row of dots.

It was noticed a few moments ago, when speaking of transposition of the bands, that at the upper focus (diag. 6, u) the coalesced bright dots form a bright band bordered at each side by a dark crenated line. Each dark line is not unlike a row of dots. Schäfer[1] seems to have figured muscle at this upper focus, and hence describes two lines of dots traversing the light disc where it borders on the dim disc.

(c) F r o g.—The fibres from the gastrocnemius of the frog treated by the same gold method as before yield an unmistakeable network. The fibres when examined are seen to be more changed by the process than is the case with insect muscle. They become very much softened and when pressed upon by the coverslip expand to many times their natural diameter, and thus often altogether loose their shape. Owing to this disturbance of the fibre the network usually shows no distinct differentiation into horizontal or transverse, and longitudinal portions. Hence there is no transverse striation.

In many places in the preparation isolated portions of fibre show a network with polygonal meshes as in fig. 7. This network is also seen at the ends of certain fibres which curling up show a transverse section. The meshes are often, when the fibre is much expanded by compression, large enough to be seen with Zeiss A. obj., at other times much smaller, approximating in size to the meshes of the horizontal networks in insects' muscle. The size of the meshes seems to depend entirely on the degree of compression of the fibre. When the

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[1] ' Quain's Anat.,' vol. ii, 9th edition, fig. 119.

meshes are small, distinct thickenings or dots are seen at the
intersections of the fibres composing the network. This net-
work is particularly sharply defined and is plainly seen to be a
true network, that is, the lines represent linear fibres only. It
is not a honeycomb work. The lines do not represent the
edges of plates of interfibrillar material.

(*d*) Crustacean. — An exactly similar network can be
brought out in the muscle of the lobster. My friend Mr. C.
F. Marshall has made preparations of lobster muscle with
acetic acid and gold which show this network in a most
beautiful manner. The muscle in this case was left in 15 per
cent. acetic acid for fifteen minutes (a much longer time than
I use), in gold chloride thirty minutes, and in 25 per cent.
formic acid in a warm chamber for three hours exposed to the
light.

This network represents the transversely and longitudinally
arranged network described in insects' muscle pulled out of
shape. In some of the fibres indeed it is still seen arranged
in the rectangular manner. Fig. 8 represents a portion of a
fibre in which transverse are crossed by longitudinal lines with
dots at the intersections. In this case the ordinary light and
dim transverse striation is obtained by refraction round the
nodal dots.

At first sight the meshes of the irregular network described
in the frog and lobster look too large to correspond in size
with the meshes of the horizontal network in Dytiscus, that is,
with the end view of sarcous elements. But we must not
forget the effect of pressure; it expands the fibre to about ten
times its normal diameter, and a corresponding increase in the
size of the meshes takes place. Fig. 11 represents a transverse
section of the fibre of the frog cut fresh with the freezing
microtome and stained by the gold method. It has not been
much enlarged by pressure and hence the meshes of the net-
work are small.

Fig. 10 represents a portion of a fibre of the lobster which
has split into fibrils; an uncommon effect in gold preparations.

When muscle splits into fibrils the fibres of the transverse net-work rupture midway between the nodal points; the longi-tudinal threads and dots remain often attached to the fibril of sarcous substance, and cause it to appear transversely striated.

The muscular fibres of the crayfish show exactly the same network, the precise method of gold staining seems to make little difference. Isolated portions of network are seen pulled out of shape, and thus with polyhedral meshes as in fig. 7. At other points the network is seen still arranged in its typical manner as in fig. 8.

(e) Rat.—In the Rat most of the fibres show the typical arrangement into transverse and longitudinal portions (fig. 9). The transverse network is most marked. In certain isolated portions the dots at each nodal point of the network are seen surrounded by bright haloes as already described.

Such then is the effect of gold staining on the muscular fibre. Can this network be demonstrated in any other way? Any method which fixes the fibre in that condition in which it is when living gives rise to appearances closely resembling those described. Acetic and osmic acids seem to act in this way.

II. Acetic Acid Preparations.

Muscular fibres from the leg of the bee were placed in dilute acetic 1 per cent. for from five to fifteen seconds, then into glycerine and mounted.

On examination they are seen to present a transverse row of dots at each membrane of Krause and longitudinal connecting rods. The network, like the sarcolemma, seems to resist the action of acetic acid more than the matrix or sarcous substance. If the fibre be stained in hæmatoxylin after the action of the acetic, the network becomes stained to a greater extent than the matrix, which remains relatively unstained.

The fibre now presents the appearance seen in fig. 15. Thin granular deeply-stained discs are seen crossing the fibre in the position of each Krause's membrane. They are attached to

the sarcolemma at the edges, and appear to divide the fibre into compartments. If the near edge of one of these discs be focussed it appears as a transverse row of dots crossing the fibre, and in many fibres fine longitudinal lines may be seen joining the dots of two adjacent discs.

In some fibres the appearance of a double row of dots crossing the fibre in the position of the transverse network is seen. This is represented in fig. 16. It is noticed in the preparations made with acetic acid, that the double rows of dots are met with, as a rule, in those fibres which have undergone least pressure. In fibres expanded by pressure a single row of transverse dots is alone observed.

Fig. 17 represents a fibre treated with acetic acid and afterwards stained in watery solution of logwood. At the upper part of the fibre the thin dotted transverse discs are not seen edgeways but partially from below. Lower down in the fibre the discs are seen more nearly edgeways, and appear in perspective view as narrow granular bands. These granular bands appear crossed longitudinally, and more or less broken up into short parallel longitudinal segments, by fine bright lines. These bright lines are caused by refraction from the longitudinal rods of the network.

III. Osmic Acid Preparations.

Preparations made by placing living muscles from the bee in osmic acid 1 per cent. for ten minutes, and mounting in balsam, give on examination the appearances figured in fig. 18 and diag. 6. Thickenings (Engelmann's " fixed waves of contraction ") are seen on many of the fibres.

In diag. 6, L the fibre is seen crossed at intervals by a dark well-marked line, Krause " membrane " or the horizontal network. On focussing upwards this line appears as a thin bright disc, and the appearance U is obtained.

In certain fibres (fig. 18), by careful examination, it can be seen that this dark line consists of a row of dots, and occasionally fine longitudinal lines may be seen joining them.

A fixed wave of contraction is shown in this figure.

The contracted part of the fibre is widened out transversely and the distance between the transverse networks diminished. The series of haloes round the rows of dots extends to the whole of the now diminished interval between the successive rows. There is consequently a bright band in the position usually occupied by the dim band. Traversing this bright band longitudinally are seen fine lines joining the dots of adjacent networks. Between this fully contracted and the relaxed part of the fibre is the portion showing the " homogeneous stage " of Engelmann. The transverse marking is here to a large extent lost, and this can be easily understood, when we consider that at the onset of contraction the transverse network would be probably more or less pulled out of shape. The individual dots would no longer lie in the same transverse plane, and hence the haloes would not blend into a continuous bright transverse disc. This agrees with the fact mentioned by Schäfer,[1] that mechanical shifting of the elements of a fibre causes a disappearance of the transverse striations.

Another point often observed in osmic acid preparations is a caving in of the sarcolemma between each transverse network, that is opposite the dim stripe. In other preparations usually the sarcolemma bulges at these points, and appears to be contracted at its attachment to the transverse network or Krause's membrane. This may be explained if it be supposed that in osmic acid preparations there is a certain amount of contraction of the matrix or sarcous substance, by exosmosis for instance. The sarcolemma will follow this decrease in bulk but will be prevented from doing so at those points where it is held outwards by the more rigid transverse networks.

IV. THE LIVING FIBRE.

The fibres from the leg of Dytiscus, or the bee, mounted without the addition of any fluid, and examined whilst fresh or living, give the appearances seen in figs. 19 and 20. Most of the fibres are seen to present the appearance of alternate dim and bright bands, the dim bands being the thicker. Each

[1] 'Quain's Anat.,' vol. ii, 9th edition, p. 129.

dim band is traversed by a series of longitudinal lines of a highly refractile substance. Running across the middle of the bright band transversely is seen a single row of dots. The fine dark lines crossing the dim stripe are traceable at either end into the dots of the bright stripe. In this case, just as in the acetic acid preparations, there often appears to be a double row of dots in the centre of the bright stripe. Fibres are seen side by side, one with a single row, another with a double row of dots in this position. When a double row is present, the corresponding dots of the two rows appear to be always joined longitudinally by fine lines across the middle of the bright stripe. This is mentioned by Haycraft[1] but not by Schäfer.

Sometimes again the appearance shown in fig. 20 is observed. A series of short parallel longitudinal lines is seen in the position of the transverse network. These lines appear dotted on careful examination. This appearance is similar to that described in the acetic acid preparation (fig. 17), and may be explained in the same way as a perspective view of the net-work crossed by longitudinal bright lines, caused by refraction from the longitudinal rods. "Transposition" of the bands may be seen on altering the focus, similar to that already described. The line of dark dots, with its series of bright haloes forming the bright disc, becomes now a line of bright dots bordered by two crenated dark lines. The above obser-vations on the living fibre were made by means of the gas chamber. The chitinous integument of the leg of the bee was slit longitudinally, the muscle scooped out, and quickly teased on a cover-glass and inverted over the moist gas chamber. This method may be used for studying the phenomena of con-traction, by blowing air charged with alcohol vapour into the chamber, and thus causing the fibre to contract by chemical stimulus.

On contraction the fibre becomes shorter and thicker, the transverse rows of dots approach one another and appear darker, probably by contrast with the now bright "dim" disc. These appearances are similar to those seen in the

[1] 'Quart. Journ. Micr. Sci.,' April, 1881, p. 23.

" fixed waves of contraction," described in the osmic acid preparations.

In a preparation of fresh muscle I have seen a fibre undergo slow rigor mortis, commencing at one end and gradually extending towards the other. It exactly resembled a very slow contraction wave passing over the fibre, and the changes undergone by successive discs, as the contraction affected them, were similar in appearance to those described in fig. 18, and could be observed with more deliberation than usual.

The Fibre under Polarised Light.—The effects observed in the living fibre with crossed Nichols were exactly similar to those figured and described by Brücke and Schäfer (' Quain's Anat.,' 9th ed., vol. ii, fig. 125). Brücke's drawing is almost identical with diagram 3.

The fibre is chiefly made up of doubly refractile or anisotropous material, but a band of singly refractile or isotropous material crosses the fibre transversely in the position of each Krause's membrane, and this band is seen with a high power to consist of a row of rhomboidal dots. Fine lines of isotropous material are described running longitudinally across the anisotropous discs and joining the rhomboidal dots. The appearance of the muscle-fibre under polarised light leads us to the belief that the network consists of isotropous, the matrix or ground substance of anisotropous or doubly refracting material.

V. ALCOHOL PREPARATIONS.

Alcohol preparations of muscle show, in most cases, a somewhat different character to those prepared by the preceding methods.

Spirit has a tendency to split the fibre into fibrils and sarcous elements. After the muscle has been in alcohol it may be stained with some reagent; Kleinenberger's hæmatoxylin, for instance, gives excellent results. Alum carmine may also be used. Mount in Canada balsam.

Absolute alcohol has a somewhat different effect from ordinary spirit. It sometimes seems to fix the fibre as

appears during life—that is, there is no differentiation into sarcous elements, but transverse rows of dots, and longitudinal lines are alone seen, as in the living fibre. Fixed waves of contraction may also be found.

Fig. 21 represents a portion of a fibre of Dytiscus stained in hæmatoxylin after the action of spirit. It shows an alternation of bright and dim discs, the dim discs stained a deep purple and made up of a series of sarcous elements side by side. Across the middle of the bright discs a dotted or granular transverse line is seen. Fine longitudinal lines, the longitudinal bars of the network, may occasionally be seen crossing the bright discs.

This account agrees for the most part with that given by Klein [1] as to the structure of muscle. He, however, figures a continuous line—the homogeneous Krause's membrane—in the middle of the bright stripe, and no longitudinal fibrillation in the bright disc.

Let us consider the influence of the intracellular network in producing the appearances known as sarcous elements, and Cohnheim's areas, in the muscle-fibre.

The matrix, or substance which lies in the interstices of the network, is of far greater bulk than the network. It is homogeneous throughout; nevertheless, it may be looked upon as being partially divided into columns or fibrils by the longitudinal bars of the network, and partially into discs—the contents of muscle compartments—by the transverse networks By the action of spirit the matrix becomes split into fibrils. The reagent causes this "sarcous substance" to shrink (possibly by abstraction of water), and the homogeneous mass now separates into fibrils along the lines of greatest weakness—that is, along the guide lines formed by the longitudinal bars of the network. These fibrils may again divide transversely at the horizontal networks, producing sarcous elements (diag. 8). Thus the appearance of sarcous elements is seen, as described by Klein,[2] to be a post-mortem phenomenon. In conse-

[1] 'Atlas of Histology,' p. 77.
[2] Loc. cit., p. 76.

quence of shrinking the sarcous substance no longer entirely
fills up the skeleton "muscle caskets," and the division into
sarcous elements, which was foreshadowed only before by the
bars of the network, becomes evident by the development of
intervening spaces between adjacent elements. The appear-
ance known as Cohnheim's areas is somewhat differently
described by different observers. For the present we may
follow Klein's[1] description. The prismatic sarcous elements
which lie side by side in the living fibre with no intermediate
substance, shrink through coagulation on dying, and become
separated from one another by a transparent interstitial fluid
substance. In a transverse view there are thus seen small
polygonal areas separated by clear lines, each polygonal area
corresponds to a sarcous element.

Cohnheim's areas may be described, as the appearances pro-
duced by coagulation and splitting of the matrix along the
guide lines formed by the transverse network; they represent
an end view of sarcous elements, and are post-mortem phe-
nomena (diag. 9).

Previous Views.

I think it unnecessary to give a historical account of the
different views which have been published with regard to the
structure of the striped muscle-fibre.

An epitome of the historical results may be found in
Schäfer's[2] paper on the leg muscles of the water beetle; or by
the same author in 'Quain's Anat.,' 9th ed.

Reference has already been made to most of the appearances
described by different observers, and the way in which these
appearances may be explained as caused by the presence of a
highly refracting network.

The relation of this network to Krause's[3] views may be
noticed. Krause's "muskel-kästchen" are bounded above and

[1] Loc. cit.

[2] Loc. cit.

[3] "Ueber den Bau der quergestreiften Muskelfaser," 'Zeitschr. f. rat.
Med.,' xxiii.

below by Krause's membrane, and laterally by the boundaries of Cohnheim's areas.   Brücke[1] regards the isotropous lines which traverse the anisotropous disc as optical sections of the partitions between muskel-kätschen.   These partitions correspond with the longitudinal bars of the network and with Schäfer's rods.   The alternation of bright and dim transverse bands has been looked upon by several observers as an optical effect, and not due to any anatomical differentiation here present.

Heppner[2] and Stricker look upon the bright band as the expression of total reflexion, which occurs at the line of demarcation between Krause's membrane and the chief substance of the fibre.

Bowman suggested that the transverse striping shown by the fibrillæ was caused by their moniliform shape.   Haycraft[3] has recently developed this view, and extended it to the whole fibre.

Striped muscular fibres are met with in the animal kingdom, from the Cœlenterata upwards; there is no reason to suppose that the cause of the transverse striation is different here from that in the insect.

I have received the greatest sympathy during this investigation from my friend Mr. C. F. Marshall, with whom I have verified most of my results.   The drawings of the network in the fibres of the Rat and Lobster are from gold preparations by him.   Mr. Marshall is at present working on the histology of the muscle-fibre, from the lowest types of the animal kingdom in which it occurs upwards, and has already obtained interesting results.   A study of the comparative development or phylogeny of this network, and at the same time of its embryology, may lead to its undoubted recognition as an ordinary intracellular network.

My thanks are also due to Prof. A. Milnes-Marshall, who

[1] 'Quain's Anat.,' p. 127 ; and "Muskelf. im polarisirten Licht," 'Wiener Denkschr.,' xv.

[2] 'Stricker's Handbook ' (Syd. Socy.), p. 548, vol. iii.

[3] 'Quart. Journ. Micro. Sci.,' April, 1881.

has kindly examined my drawings and specimens, and suggested alterations in the paper, and to Mr. J. Priestly, M.B.

Brief Summary of Results.

The chief results at which I have arrived may be summarised as follows :

There is an intracellular network present in the striped muscle-fibre of Dytiscus, the Bee, Frog, Lobster, Crayfish, and Rat, which may be most clearly demonstrated by certain methods of gold staining. The network alone is stained by the reduced gold, and, owing to this differentiation, is plainly visible even with comparatively low powers. This network may be demonstrated, though not so completely, in the living fibre, and in acetic and osmic acid preparations.

Crossing the fibre transversely, united to the sarcolemma, and more or less separating the muscle-fibre into compartments, are network partitions—the transverse networks.

Running longitudinally down each compartment, and joining the dots at the intersections of the fibres of the transverse network, are a series of fine rods. The arrangement of this network will be made evident by reference to diagrams 1, 2, 3, and 4.

This network consists of an isotropous material, and is more highly refractile than the rest of the muscle substance, which is anisotropous. This network serves to explain the transverse striation and other complicated appearances presented by the muscle-fibre, and brings into harmony many of the conflicting statements of histologists on this subject.

DESCRIPTION OF PLATE XXIV,

Illustrating Mr. B. Melland's Paper on " A Simplified View of the Histology of the Striped Muscle-Fibre."

Diag. 1, 2, 3, and 4.—Diagrammatic view of the network in striated muscle.

Diag. 1. Perspective view of the fibre, showing the transverse network *a* at each membrane of Krause, and the longitudinal lines.

Diag. 2. Perspective view of a portion of the network, showing:—*a*. The transverse networks, with polygonal meshes and dots at the nodes. *b*. The longitudinal bars of the network ending in the dots.

Diag. 3. The fibre seen in longitudinal view. The transverse network, *a*, appears as a row of dots crossing the fibre (in the position of Krause's membrane). *c*. Minute thickenings on the longitudinal bars of the network, midway between the transverse networks.

Diag. 4. The fibre seen in transverse section.

Diag. 5 —Network as seen in a longitudinal view of the fibre, showing the production of alternating bright and dim bands by refraction around the nodal dots.

Diag. 6.—So-called transposition of the bands, as seen in an osmic acid preparation of muscle of Bee. u. Appearance at upper focus. l. Appearance at lower focus.

Diag. 7.—Oil globules in water, showing their refractive effect upon light. u. At the upper focus, each globule surrounded by a dark border. l. At the lower focus, each globule surrounded by a bright halo.

Diag. 8.—Production of sarcous elements by contraction of the matrix and splitting along the guide lines formed by the bars of the network (seen in spirit preparations).

Diag. 9.—Formation of Conheim's areas by contraction of the matrix as above. In this transverse view of the fibre the prismatic sarcous elements are seen on end, and appear as polygonal areas separated by bright lines.

Fig. 1.—Fibre of Dytiscus, prepared by the gold method. Zeiss, D obj., No. 5 oc.

Fig. 2.—Dytiscus, gold method, portion of a fibre more compressed than in Fig. 1.

Fig. 3.—Fibre of Bee, prepared by the gold method ; transverse networks in perspective.

Figs. 4 and 5.—Dytiscus, gold method, showing isolated discs consisting of a network.

Fig. 6.—Fibre of Dytiscus, gold method, splitting into discs.

FIG. 7.—Lobster fibre, gold chloride; isolated portion of a fibre, network pulled out of shape. Exactly similar networks are seen in the Frog and Crayfish.

FIG. 8.—Frog, gold method; network arranged typically, and showing transverse striping.

FIG. 9.—Rat, gold chloride; longitudinal view of portion of a fibre. (Preparation by C. F. Marshall).

FIG. 10.—Lobster, gold chloride, splitting into fibrils.

FIG. 11.—Frog. Transverse section of the frozen fibre, stained by the gold method.

FIG. 12.—Dytiscus, gold method; isolated portion of the network.

FIG. 13.—The same, more highly magnified. (Zeiss, F obj , No. 5 eyepiece.)

FIG. 14.—The same, showing refracting effect of the network.   L. Lower focus.   U. Upper focus.

FIGS. 15 and 16.—Fibres of Bee, treated with acetic acid, then Kleinenberg's hæmatoxylin.

FIG. 17.—Fibre of Bee, treated with acetic acid, then watery solution of logwood. The transverse networks seen more or less obliquely.

FIG. 18.—Fibre of Bee, prepared with osmic acid, shows a fixed wave of contraction.

FIG. 19.—Living fibre of Bee, showing longitudinal view of network ($\frac{1}{15}$ immersion obj).

FIG. 20.—Living fibre of Bee, transverse networks seen somewhat obliquely.

FIG. 21.—Portion of a fibre of Dytiscus, stained in hæmatoxylin after the action of spirit. Shows sarcous elements.

Where not otherwise stated, the drawings were made from Zeiss, D. obj., No. 5 occ.
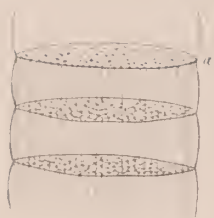
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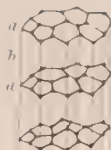
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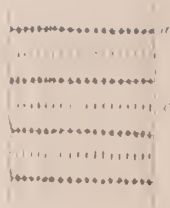
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Diag. 8.

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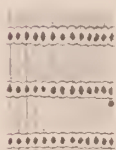
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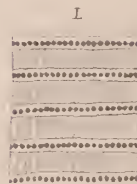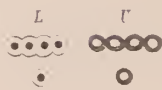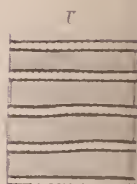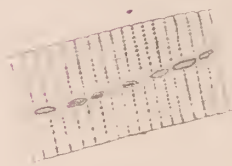
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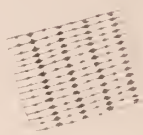
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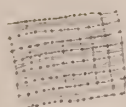
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Fig 10

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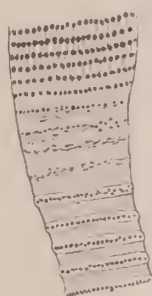Fig. 12.

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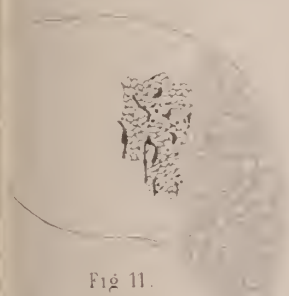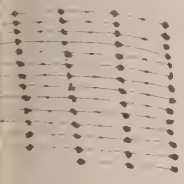
Fig 14

Fig. 15.
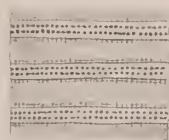
Fig 16
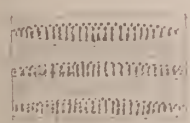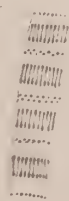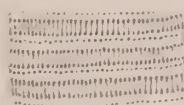
Fig. 17.

Fig. 18.

Fig. 19.

Fig 20

Fig. 21.

On the Development of a Freshwater Macrurous Crustacean, Atyephira compressa, De Haan.

By

Chiyomatsu Ishikawa,
Of the University of Tokio, Japan.

With Plates XXV, XXVI, XXVII, XXVIII.

THE species on which the following observations were made, is very commonly found in freshwater streams and ponds in the vicinity of Tokio. Its specific determination has very kindly been made for me by Dr. Ed. J. Miers, of the British Museum, through the kind offices of Professor Whitman and Dr. Paul Mayer. Dr. Miers wrote a valuable paper on it, in the 'Annals and Magazine of Natural History' for March, 1882 (pages 193, 194).

My investigations on the development of Atyephira compressa, De Haan, were begun in the spring of the year 1881, under the direction of Professor Whitman, to whose valuable assistance and instruction I am deeply indebted. By the spring of the next year I had nearly completed the study of the development of the ovarian ovum, and on my graduation in July of that year, wrote a thesis on this subject under the name of "On the Ovarian Ovum of Atyephira compressa, De Haan." In the spring of 1883 I extended my investigations to the development of the post-ovarian ovum, under the direction of Professor Mitsukuri, to whose constant

advice and never-failing encouragement I am very much in-debted.

To the authorities of the University of Tokio, and especially to the President, Mr. H. Kato, I am deeply indebted for the use of the instruments, chemicals, and other things necessary for pursuing my work.

I must here also express my great obligation to Dr. Faxon, for sending me ' The Memoirs of the Museum of Comparative Zoology at Harvard College,' vol. ix, No. 1, which was of great use to me.

METHODS.

For the dissection of the ovary, I have used Zeiss's Dissecting Microscope, with a magnifying power of about fifteen diameters.

For examination of fresh specimens :

a. Ovaries were examined in bicarbonate of potash or of ammonia 2 per cent. strong. Connective tissues were treated with acetic acid ($\frac{1}{2}$ per cent.) and coloured with aniline.

b. Embryos were removed from the yolk mass by means of needles in the normal salt solution, and exposed to the fumes of osmic acid (·05 per cent. strong) for about five minutes.

For the surface view of the ovarian walls, nitrate of silver was always employed.

For sections embryos were hardened in Kleinenberg's picro-sulphuric or Mayer's picro-nitric acid for about three hours, and passed through successively weak, strong, and absolute alcohols. They were then stained in the logwood-solution or borate of carmine. In the former fluid the yolk-spherules were coloured magnificently, while in the latter they remained uncoloured.

Books of references are noted throughout this paper by the use of the Arabic numerals. The denominators in the frac-tions refer to the list of authors at the end of this paper, and the numerators indicate the page. There are, however, many works to which I could not get an access. These are referred to, as far as possible, by citations found in those books at my command. Such works are noted in the list by an asterisk.

An interrogation point used in a fraction indicates that the pages are unknown.

The Ovary.

The female generative organ of Atyephira compressa consists of two elongated sacs joined with each other near the anterior end.   They extend in the body cavity parallel to, and above, the digestive canal, beginning at the middle portion of the cephalothorax, a little in front of the heart, and reaching posteriorly as far as the middle of the second abdominal segment.   At about the middle point of its length each of these sacs sends out a canal laterally, which taking a direct course downwards, opens on the internal face of the basal joint of the third thoracic leg.   The mouth of this canal is covered over by a membrane which is slightly raised above the level of the general surface.   There is a transverse slit across the middle of this membrane, which thus forms a sort of a valve, and prevents water from getting into the ovary (fig. 1, *o.od.*).   The elongated sacs above mentioned constitute the essential part of the ovary, while the two lateral ducts serve as oviducts.

The fully ripened ovary (fig. 2) measures about 4.5—6 mm. in length and 4 mm. in breadth across the point of junction. These measurements, however, vary according to the size of the animal, for an animal not larger than 10 mm. in length is often provided with a small ripe ovary, containing a small number of eggs.

On the under side of each ovarian tube there is to be seen a narrow transparent band, sharply distinguished from the dark green colour of the ripe ova (fig. 2, *ger.*), running the whole length of the ovary.   Anteriorly it runs nearer to the median than to the outer side.   It meets with its fellow of the opposite side at the point of junction, whence it takes its course diagonally outwards to the region of the oviduct in a curved line ; here it turns posteriorly, and taking its course along the outer edge of the ovarian tube, ends blindly at its hind extremity.   These bands are very well seen when the ovary is acted on by acids

such as chromic, picro-sulphuric, or nitric. They appear dis-
tinctly as white bands on the now orange-red (with chromic)
or reddish-yellow (with picro-sulphuric or nitric) portion. They
represent the formative place of the eggs, and are filled with
young ova that have not yet acquired yolk-elements. I desig-
nate these bands under the name of " germogen " inasmuch
as it is in these parts that the primordial ova are found. The
rest of the ovary will be spoken of as " vitellogen," where the
vitellus or the yolk-elements are formed.

A section of an ovary (fig. 3) will therefore show two
distinct groups of eggs, one consisting of larger and the other
of smaller eggs. The former (fig. 3, *Vit.*) fills up nearly the
whole cavity of the ovarian sac, while the latter (fig. 3, *ger.*)
occupies only a small portion. This latter represents the
white band shown on a surface view (fig. 2, *ger.*).

The section represented by the fig. 3 is a transverse one cut
through the posterior portion of the ovary, the smaller group
of eggs occupying the outer side of the tube, and the larger
the inner.

This arrangement seems to be almost universal in the crus-
tacean ovary. The sexual organs of Amphipods have been
studied by Bruzelius, Spence Bate, and de la Valette St.
George. They have all described these organs as cylindrical
tubes, whose peripheral parts are occupied by young eggs, while
the central portions are filled with much more advanced eggs.
Of the Isopod ovary Leuckart says : " The ripened eggs always
take the whole inner side of the egg sac; the youngest, on the
contrary, lie on the outer side in its entire length. Only the
outer side is the formative place of the eggs." Edouard van
Beneden, in speaking of the Amphipods and Isopods together,
expresses his views in the following words ($\frac{130}{1}$):—" In all
these animals there must be distinguished throughout the
length of the ovary two very distinct parts; the one situated
on the external side presents itself under the form of a narrow
band, and filled with young eggs at different stages of
development."

The Ostracod ovary is stated to be of a similar nature ($\frac{99}{2}$).

Among Decapods we find it in Eupagurus, Crangon, Panulirus, Atyephira, and so on. Mayer's description of the ovary of Eupagurus corresponds with that of Atyephira in almost every point ($\frac{1}{3}\frac{9}{3}^3$). In our common Crangon (probably C. vulgaris, Fabr.) the white band takes its peripheral position on the anterior half of the ovary, while posteriorly it penetrates into the interior of the ovary, and takes the axial course. In Panulirus japonicus, Gray, I have found it taking the axial course through the whole length of the ovarian tubes.

Structure of the Ovarian Walls.—The wall of the ovarian tube consists of two sets of layers, the outer and the inner, more or less separated from each other. The outer set is formed of (1) a connective-tissue layer, and (2) a superficial epithelium layer; the inner consists of (1) a fine structureless membrane, and (2) a layer of pyramidal epithelial cells. The outer connective-tissue layer (fig. 4) is formed of a thin matrix, embedded in which are seen fine, wavy, parallel lines, seemingly marked out into imperfect fibres, running in no one definite direction, but, on the whole, lengthwise. The nuclei of this layer (fig. 4, nc.) are very variable in sizes, measuring 0·013—0·01 mm. in length, and 0·006—0·01 mm. in breadth. Their forms are somewhat flat and elliptical. They have very delicate contour, and are provided generally with nucleoli, which appear as minute dots. We often recognise in this tissue the presence of pigment patches, whose form and size vary exceedingly. The colour of the pigment varies also from yellow to dark carmine. Clear spaces or lacunæ of variable dimensions are generally to be seen intermixed with nuclei.

This connective tissue can best be studied, as I have already said, in fresh specimens treated with very dilute acetic acid (·005 per cent.), and coloured with aniline or carmine, or else by simply colouring the freshly-taken ovary with Beale's carmine. By the former method the nuclei appear to be a little larger than they should, being (most probably) swelled up by the action of acetic acid.

This connective-tissue layer extends all over the ovary and

oviducts, connecting the two ovarian tubes together, and it also covers over other organs—the heart, the alimentary canal, &c.—and therefore may not properly be said to belong to the ovary. A similar layer of the connective tissue is stated to be present in the ovaries of Isopods and Amphipods.

Below the connective-tissue layer comes a layer of epithelium cells. This layer (fig. 5) consists of cells with irregular outlines, of about 0·03 mm. in diameter, provided with an oval nucleus of about 0·01 mm. in length. It extends all over the ovary and oviducts. The cells on the latter are much smaller than those on the former, although the nuclei are nearly of the same dimensions (compare figs. 5 and 6). The nuclei are very difficult to make out, even in silver staining. In fine preparations, however, they appear to be provided with one or more very small dots—the nucleoli.

This epithelium is sometimes (perhaps always) followed by another sheet of a cellular layer, apparently similar to it in structure, differing, however, from it in its being turned in, sometimes, though rarely, between the egg follicles. This cellular layer I am inclined to consider as akin to the ovarian stroma of other animals.

Between this and the inner set of layers a space (figs. 7 and 8) occurs filled with finely granular fluid, in which clear nucleated cells (*b c*) are to be discerned. This space is not continuous all around the ovary. It remains always uncoloured, or only very slightly coloured by various staining fluids, and appears of a dirty yellow hue. Of the cells floating in it the nuclei and the nucleoli colour very deeply, while the cell body always remains uncoloured. They have a very delicate but definite contour. By these and other peculiarities they are easily distinguished from all the other cells found in the ovary. They are sometimes found solitary, when they have a round or oval form, but often in groups, when they are polygonal. Sometimes in narrow spaces they arrange themselves in compact rows of square cells. This granular mass evidently represents blood plasma and the cells blood-corpuscles.

I made many attempts to inject the ovary with some

colouring fluid, and thus to bring out the nature of these spaces clearly, but unfortunately I have not succeeded in doing so.   Still I think I have enough reason for believing that they are blood spaces, and nothing else ; for in the first place the sections of the heart and blood-vessels show us that in their cavities the same granular mass with similar cells occur.   The granular substance and the cells exhibit no difference whatever to those found in the ovary.   In the second place, blood taken out of the body of the animal, and exposed to the fumes of osmic acid 0·5 per cent. strong for about five minutes, and coloured with Ranvier's picro-carmine, shows the blood-corpuscles exactly corresponding in shape and size to those found in the ovary.   In the third place, the above-mentioned cells are extremely variable in form, and have no definite position in the granular mass.   In the fourth place, the ovary of Panulirus japonicus, Gray, whose blood-vessels have been injected with a blue injecting mass, shows the presence of blood in similar places.   Finally, fresh specimens show blood-corpuscles in the corresponding place.

Blood not only gets into these spaces, but enters into the vitellogen, and fills up the spaces or lacunæ between the adjoining eggs (figs. 3 and 13 *bs.*), while in the germogen no trace of them can be detected.   These blood spaces are best observed in the ovary not fully developed.

Of the distribution of the blood vessels in the ovary, I have not been able to make a satisfactory observation in Atyephira ; but I have made it out clearly in Panulirus, of which I may therefore be allowed to write a few notes here.

The ovary of Panulirus japonicus, injected with the blue injecting mass, showed me that the sternal artery gives off a large branch to the right ovarian lobe.   It soon divides into two branches, of which one, running anteriorly for a short time, sinks deep into the body of the ovary, while the other runs through the entire posterior lobe.   Slightly anterior to the origin of the sternal artery, a small branch is given off to the left lobe directly from the lower side of the heart.   This runs posteriorly for a short distance, but soon divides into two

branches. Like that to the right lobe, one of these taking the
backward course runs through the entire posterior part, while
the other running anteriorly, soon sinks into the substance of
the ovary.

In front of the heart two other branches are given off, from
the antennal arteries. Each of these soon divides into three
main branches : the anterior, the posterior, and the median or
lateral. The anterior runs along the whole length of the
anterior half of the ovary, the posterior divides into fine
capillaries, and penetrates into the interior of the ovary, while
the lateral unites with its fellow of the opposite side. There
are no large vessels on the lower side of the ovary.

In some specimens, the sternal artery runs over the left lobe
of the ovary, giving off a branch to it, while the right lobe
was supplied with one given off directly from the heart.

The inner set of layers in the ovarian walls consists of (1) a
fine structureless membrane (fig. 10, $mb.$), and (2) a layer of
pyramidal epithelial cells. This epithelial layer will be spoken
of as the "internal" epithelial layer in contradistinction to
the one already described, which will be called the "external."
The internal epithelial cells exist only over the vitellogen, the
walls of the germogen being devoid of the epithelium properly
so called. The epithelium cells (figs. 8, 9, and 10, $fe.$) are in
general somewhat elongated, and are furnished with a finely
granular nucleus, in which a nucleolus can be detected. The
cells measure about 0·2 mm. in length and 0·08 mm. in
breadth, and the nuclei are about one tenth the size. The
epithelium, together with the structureless membrane, enter
into the ovary and surround the eggs in the vitellogen, form-
ing thus a sort of a follicle (figs. 8, 9, 10, 13, $fe.$).

The wall of the oviduct shows the same structure as that of
the ovary itself, excepting that the superficial epithelial cells
are, as already stated, decidedly smaller than on the ovary
proper, being only about one third.

It appears thus that the wall of the ovary and oviducts
consists (1) of a connective-tissue layer; (2) of an external
epithelium ; (3) of stroma ; (4) of blood space ; (5) of a struc-

tureless membrane, on the inner side of which is (6) a layer of internal epithelial cells.

The Formation of Eggs:—In the white band already spoken of (figs. 2 and 3, *ger.*), we find the youngest eggs. They appear perfectly clear and transparent, and measure about 0·01 mm. in diameter, with roundish nuclei of about 0.008 mm., the size of the nuclei of the inner epithelial layer. A section of an unripe ovary, whose lobe measures about 0·14 mm. in cross diameter (fig. 9), shows us that the ovary is surrounded by a single-layered wall. This wall corresponds to the external epithelial layer of a ripe ovary. The sac is filled with a general mass of nucleated cells embedded in protoplasm, which latter shows a fine granulation in prepared specimens. The wall of these cells is extremely delicate, but is clearly distinguishable in fine preparations. Some of these cells probably arrange themselves around the inner side of the ovarian wall as the internal epithelial layer, some surround the larger eggs that have moved to one side of the ovarian tube and become the follicular (?) epithelium (fig. 9, *f. e.*), while the others grow into primordial ova (*p. o.*). Thus at this stage of the development of the ovary there is no marked difference to be observed between the cells that grow into eggs and those that become the internal epithelial or follicular cells.

In the section of a larger ovary we find the youngest ova all along the external side of the germogen immediately below the external epithelial layer. But the interesting stages are represented in those sections (figs. 10 and 11), cutting through the oviducts, whose internal epithelial cells (*i. e. od.*) are seen to acquire a more rounded form (*p. o.*) and pass uninterruptedly into small eggs. Mayer ($\frac{193}{3}$) affirms that a genetic relation exists between these two elements. Waldeyer in his "Eierstock und Ei" ($\frac{85}{4}$) mentions a similar case in the ovary of Astacus fluviatilis. Of this last animal Professor Huxley also says ($\frac{132}{5}$) : "The growth of these cells gives rise to papillary elevations which project into the cavity of the ovary and eventually become globular bodies attached by short stalks, and invested by the structureless membrane as a membrana

propria. These are the ovisacs. In the mass of cells which becomes the ovisac, one rapidly increases in size and occupies the centre of the ovisac, while the others surround it as a peripheral coat. This central cell is the ovum." If we conceive a number of such ovisacs to arise in a special part of the ovary, and joined with one another in a line longitudinal to the ovary, we shall have a case somewhat similar to that of Atyephira, from which, however, it differs in the fact that only one, out of a number of cells that constitute an ovisac, grows to be an ovum. In Atyephira, as we have already seen, all of the cells, or the majority of them, no doubt, in the pouch are destined to become eggs. It will be observed further, that a complete distinction is here made between the cells which constitute the follicular epithelium, and those which become eggs.

The youngest eggs (figs. 9, 10, and 11, *p. o.*) have their size equal to those of the epithelial cells. They have a very delicate contour, and their germinal vesicle generally contains one or two germinal dots. They are quite transparent until they grow to the size of at least ·09 mm. in length. In a little older eggs, the protoplasm, however, shows a very delicate tint of blue, while the germinal vesicle appears of a very faint ochre. There is also present in the ovum one or more vacuolar spaces (figs. 10, 11, 13 and 14, *vac.*). They appear in the ovum of about ·035 mm., and their number rapidly increases when the ovum is transferred into the vitellogen. Ed. van Beneden has observed beautiful amœboid movements in very young eggs of Isopods, Amphipods, and some Decapods— Crangon vulgaris—when they are held in suspension in the serum of blood or in aqueous humour. My experiments have failed to detect such a phenomenon in the eggs of Atyephira.

The germinal vesicle is at first uniformly granulated by fine granules, provided with one or more germinal dots, which latter can only be distinguished from the granules by their larger size, and by their being strongly coloured by staining fluids (figs. 10, 11, and 12). As it grows in size these granules become coarser, and patches of stellate figures (figs. 13 and 14) are formed, probably by the union of the granules.

The size of the germinal vesicle is proportionately larger in young eggs than in the older ones, the growth of the vesicle being slower than that of the egg. Their proportionate sizes are to be seen from the following :—

Egg.		Ger. Ves.		Egg.		Ger. Ves.
·01 mm.	.	·007 mm.		·028 mm.	.	·014 mm.
·011 ,,	.	·009 ,,		·05 ,,	.	·02 ,,
·013 ,,	.	·009 ,,		·052 ,,	.	·028 ,,
·014 ,,	.	·0095 ,,		·068 ,,	.	·03 ,,
·016 ,,	.	·01 ,,		·08 ,,	.	·035 ,,
·017 ,,	.	·01 ,,		·085 ,,	.	·03 ,,
·018 ,,	.	·01 ,,		·09 ,,	.	·03 ,,
·020 ,,	.	·012 ,,		·095 ,,	.	·035 ,,
·021 ,,	.	·012 ,,		·160 ,,	.	·045 ,,
·024 ,,	.	·013 ,,		·350 ,,	.	·07 ,,
·026 ,,	.	·013 ,,				

It is clear from this that while the germinal vesicle is at first more than one half, it is only one fifth when the egg attains the diameter of about ·35 mm. In Eupagurus, Mayer mentions a similar case, where he found the germinal vesicle of 44 μ in the egg of 72 μ, and soon after the germinal vesicle of 62μ in the egg of 146 μ ($\frac{124}{3}$).

The germinal vesicle is provided with one or more germinal dots, but never more than three. They generally take an excentric position, and are, in the first stages of their development, simply aggregations of the protoplasmic granules in the germinal vesicle ; which gradually coalesce into a form of considerable dimension (fig. 12). This coalescence takes place either at a single spot, when only a single dot is formed, but often at two or three different places, when we have two or three germinal dots. At this stage of development, which is represented by the egg of, at the most, ·02 mm., they have no definite boundary, but as they grow in size, the granulations seemingly fuse together into a roundish mass, having a smooth edge. At the same time, a number of vacuoles appear in them, which, however, dwindle away later.

When the eggs grow to the size of at least ·10 mm. in

diameter, they pass into the vitellogen, to be charged with nutritive elements. Here they grow very rapidly, and their colour gradually becomes altered to a dark green. Nutritive elements or yolk-spheres grow and multiply rapidly on all points of the egg, but especially in a region near the periphery. The vacuolar spaces, mentioned above, also increase very rapidly in number and size, while the protoplasm becomes thus alveolar in its structure (fig. 14). The protoplasm which had originally filled the entire body of the ovum, has now become very scarce; i. e. in proportion to the size of the egg; and when the egg attains its full dimensions, it becomes a matter of great difficulty, even in nicely prepared sections, to discern the presence of protoplasm among thickly crowded vacuoles and yolk-spheres.

This peculiar arrangement of protoplasm suspending the deutoplasmic elements in its meshes, is also stated to occur among Vertebrates ($\frac{4.9}{7}$).

But how, it may be asked, do the yolk-spheres of the egg originate? Do they develop in the protoplasm of the egg or do they arise from the investing follicular cells? Lereboullet ($\frac{1}{8}$) regards them as originating in a special kind of cell containing the yolk substance, while Waldeyer ($\frac{8.5}{4}$) derives the yolk-elements from the follicular epithelium cells. Ed. van Beneden ($\frac{7}{1}$) says: "I have believed, at first, that the nutritive elements of the vitellus had taken birth in the special cells of the vitellogen, and that these cells are absorbed by the protoplasm of the egg-cell. But I have soon recognised that there is a considerable error, and that the germs never present the cellular appearance, if we observe them in the interior of the sexual utricle or in an indifferent fluid, such as the iodite of serum or a solution of albumen. At present I am certain that the nutritive elements of the vitellus always form themselves in the interior of the protoplasm of the egg-cell, as Mr. de la Valette St. George has recognised long ago." In Astacus fluviatis, according to Professor Huxley ($\frac{1.3.3}{5}$), "the protoplasm of the cell, as it enlarges, becomes granular and opaque, assuming a deep brownish-yellow colour, and is thus converted

into the yolk or vitellus." I believed for a long time that the nutritive elements were derived from the vacuolar spaces already mentioned, because in these spaces I have often observed, in fresh specimens, small refracting spheres which resemble very much those of the yolk-elements. I have, however, found out that my so imagined yolk-spheres were nothing but the particles of water that have, in some way or other, found their way into these vacuolar spaces; for when I examined the ovary in the solution of bicarbonate of potash or ammonia (2 per cent. strong), I have invariably found that the vacuolar spaces are free from any such thing, and that these elements are only seen when the ovary is examined under water.

As the egg increases in size the protoplasm becomes coarsely granular at the periphery. These granules are quite opaque at first, but become more or less transparent as they grow in size; they assume a deep greenish colour and become converted into the yolk-spheres. The yolk-spheres are in general round and homogeneous, showing no trace of a nuclear structure. Their size varies much, the smallest being no larger than yolk-granules, and the largest often measure ·003 mm. in diameter. My observations therefore point out that the yolk-spheres originate endogenously out of the protoplasm of the egg.

This agrees with the statements made by Huxley in his Crayfish ($\frac{133}{5}$), and still more with the results obtained by Professor Whitman in his Clepsine ($\frac{222}{6}$).

As the yolk-spheres and the vacuoles are formed the protoplasm of the egg becomes reticular in its structure, suspending these spherules in its meshes. But at two places the protoplasm remains in a more or less thick continuous layer. These are around the germinal vesicle, and at the extreme periphery of the egg. The peripheral protoplasm appears to give origin to a distinct membrane, which I call by the indifferent name of "primary egg-membrane." In the oviduct it receives another membrane from the lining epithelial cells, which at this time present a glandular appearance (fig. 15). This outer membrane will be spoken of as "secondary egg-membrane." Between

these two membranes a certain quantity of clear liquid is found, which coagulates and becomes finely granular in alcohol.

The primary egg-membrane always shows a granular reticulated appearance, with oval or round interstices. These are caused by the yolk-spheres, on which the membrane closely adheres at the beginning, and is not to be mistaken for the cellular markings found in insect ova. The secondary egg-membrane is perfectly structureless, and is thicker and firmer than the primary. Both egg-membranes are at first extremely elastic, as is seen in the exit of an egg through the narrow opening of the oviduct.

A number of different observers have spoken of the existence of two membranes on the eggs of Decapods. Rathke ($\frac{6}{9}$) and Reichenbach ($\frac{127}{10}$) have found them in the eggs of Astacus fluviatilis, Bobretzky in Palæmon, Erdle in Homarus, and Dohrn in Scyllarus arctus ($\frac{251}{11}$), Palinurus vulgaris ($\frac{250}{11}$), and in Portunus ($\frac{618}{11}$).

It remains now for me to consider very shortly the germinal vesicle and its final fate. The germinal vesicle presents one or two or rarely three germinal dots in younger eggs; but in those eggs which are grown to a considerable size, and which are found in the vitellogen, are always provided with a single dot. It is thus distinguished from the ova of Astacus, in which Lereboullet, Waldeyer ($\frac{85}{4}$), Huxley ($\frac{133}{5}$), and others have found many germinal dots, and from that of Eupagurus, where Mayer has found only a single dot in all the stages of the development of the egg. It does not grow, as I have already mentioned, as rapidly as the egg, and when the egg is quite ripe the germinal vesicle disappears. I have made hundreds of sections of the ripe ovary, and have always found the ripened eggs devoid of nucleus, while the younger ones show it very plainly. No nuclear structure can also be seen in freshly-laid eggs. Mayer speaks of it so explicitly in the egg of Eupagurus that I will quote his own words here ($\frac{199}{3}$) :—
" Nach einiger Zeit, und zwar noch während das Ei im Ovarium befindlich, verschwindet—wie dies auch schon Rathke von Astacus angibt—das Keimbläschen, so dass die frisch

gelegten Eier positiv kernlos sind." But how it dwindles away is a question not yet decided. The germinal vesicle, which is at first always in the centre, is often found to be somewhat excentric in position in an egg little advanced, but never at the periphery. Commonly, however, I have seen the wall of the vesicle indented and rather indistinct. Whether this shows the transitional stage from the nucleated cell to a cytode or not I cannot venture to affirm. All that I can say is that the germinal vesicle disappears while the egg is still in the ovary.

To sum up, then—

1. The ovarian egg of Atyephira compressa originates from the inner lining epithelium of the ovary, and is, at the beginning, a cell with a nucleus, and one, two, or rarely three, nucleoli. Later, the deposition of the yolk takes place endogenously.

2. The protoplasm of the egg collects at two points, the one around the nucleus and the other at the periphery. The former spreads out like rays towards the latter, and unites with it.

3. The germinal vesicle grows less vigorously than the egg. It disappears speedily when the egg attains a certain size.

4. The ripened egg is covered by two membranes, the one formed by the hardening of the peripheral protoplasm of the egg, while the other is formed by the product of the epithelial cells of the oviduct. Between these two membranes a certain quantity of a clear transparent liquid is found.

5. The freshly-laid egg is unfurnished with a nucleus, and is therefore a cytode.

Laying of Eggs.—When the eggs are fully grown, and ready to be laid, the region of the oviduct appears white by the reflected light through the carapace. The section of such an epithelium will show that the lining cells are very much elongated (fig. 15). If an animal in such a condition is put into a vessel and watched for a day or two the laying of eggs can easily be observed. This generally takes place in the early

morning, and is preceded by an exuviation, which usually occurs
during the night.   The mode of egg-laying seems to be essen-
tially the same as that described by Lereboullet, although, un-
fortunately, I have not been able to get an access to his original
description, an inconvenience which often happens to a natu-
ralist working here.

The prawn before the egg-laying is seen to be very uneasy
(the uneasiness may, perhaps, be owing to the late exuviation),
continually moving about until it finds a good resting place.
It then bends its body downwards in the form of a fish-hook,
and thus forms a sort of a pouch with its abdomen, the tail of
the animal corresponding to the point of the hook.   Into this
pouch the eggs are laid.

During the act of laying the thoracic legs are kept continually
moving, the last pair seemingly assisting to drive the eggs
downwards, while the swimmerets are seen to be in rapid
motion.   The abdominal segments from the first to the fifth
are also seen to move rhythmically.

The eggs in coming out of the oviduct become very much
elongated, almost rod-like, and, outside of the body, seem to
take their course along the median line until they reach the
abdomen, where they stick to the swimmerets.   As regards
the position of the eggs in relation to the swimmerets, I have
observed that those eggs which come out earlier are received
by the anterior pairs, while the later ones are driven to the pos-
terior by the last pair of thoracic legs.   As soon as they leave
the oviduct they become more spherical, until they take their
characteristic ellipsoidal form.

There seem to be various opinions as to the means of the
fixation of the eggs to the hairs of the swimmerets.   Accord-
ing to Lereboullet, the soft skin of the abdomen of the mother
crayfish (Astacus fluviatilis) secretes a liquid which gives
origin to both the outer egg-membrane and to the substance
binding the eggs to the swimmerets.   Huxley in his Crayfish
says ($\frac{40}{5}$) : "These as they leave the apertures of the oviducts,
are coated with the viscid matter, which is easily drawn out
into a short thread."   For my own part, I can say almost nothing

on this point except that I have not seen any gland other than those found in the oviducts.

At an early period of development, the inner and the outer egg-membranes lie so closely together that it is a matter of great difficulty to separate them. They are both very elastic and are quite structureless, except that the inner egg-membrane is marked with the polygonal areas already spoken of. The newly-laid eggs show no structure like a nucleus in them. I have often tried by means of sections and otherwise to find out how the nuclei of the post-ovarian eggs arise, and how the spermatic elements act upon them, but I have entirely failed, as I did in the case of the disappearance of the nuclei. All that I am certain of is that the original nuclei disappear before the formation of fresh ones capable of segmentation, and that the egg probably receives the male elements as soon as they come out of the oviducts, for the reason that I have often observed a spermatophore attached on the sterna of the female during the breeding season.

Judging from figs. 14 and 15 represented in Faxon's "Embryological Monographs," pl. iv, I see that the male and the female pronuclei were found in the eggs of a Copepod (Cetochilus septentrionalis) by Grobben. In sections of freshly-laid eggs I have twice observed an appearance that may be interpreted as the process of the fusion of these two elements; but this I can say only with much caution, for I have never seen the stages before or after it.

The form of the egg is in general ellipsoidal, measuring about 0·75—0·85 mm. in long axis and 0·45—0·55 mm. in short axis. Its colour varies much according to the colour of the mother prawn, but is usually of a yellowish green. The colour of the animal varies again with the surrounding objects; thus when the prawn is caught among the green grass it is more or less greenish, but when it is caught among the dead grass, which is usually the case in the late autumn and in winter, it partakes somewhat of the colour of hay. When, again, the animal is transferred from its natural habitat to a white dish, it gradually loses its colour in the course of a few

days, until it becomes nearly colourless. This also seems to be the case with the eggs; undergoing similar changes, when they are subjected to different external conditions. A case of similar nature is also stated to occur in the eggs of Eupagurus by Dr. P. Mayer where he says $(\frac{210}{39})$: "Es bestehen aber in der Intensität der Farbe individuelle Abweichungen, wie dies auch schon vor mir andere Autoren beobachtet und mit seltener Uebereinstimmung auch stets (ob mit recht?) auf Verschiedenheiten in der Färbung des Mutterthieres zuruckgeführt haben."

DEVELOPMENT.

Segmentation:—The newly-laid egg shows no nucleus, as before remarked, but soon a definite one appears in the centre. The inner and the outer egg-membranes, which at this early date lie so closely together, separate after a lapse of ten to fifteen hours, and a certain quantity of albuminous fluid collects between them. A similar fluid is stated to occur in the Orchestia eggs by Ulianin $(\frac{441}{13})$, where, however, the occurrence is of a much later date, namely, at such stages where a fine cuticular skin becomes distinguishable around the embryo. This fluid becomes coarsely granular when the egg is treated with such reagents as alcohol, acids, &c. It becomes a cause of great hindrance for hardening, as has already been pointed out by Ulianin $(\frac{441}{13})$; and in order to avoid this difficulty I have used the same method described by him, by which means alone I was enabled to harden the eggs properly. This method consists in breaking off the secondary egg-membrane by means of the points of needles under the dissecting microscope, the eggs being placed in a watch-glass under water. After the greater part of the albuminous fluid has escaped out of the slit thus made, the eggs were brought to the hardening fluid, which now reaches the embryo easily.

Segmentation begins by a slight notch on one side of the egg transverse to the long axis (figs. 17 and 18). This notch gradually elongates both ways, until the egg is divided into

two equal parts (figs. 19 and 20). After remaining in this condition for about two or three hours, the segmentation line becomes narrower, the two halves gradually approaching each other, and at last the egg is again in a condition not externally different from that which we started (fig. 23). Internally, however, we see the nuclei clearly separated from each other, and these are sometimes in the state of division, showing thus the commencement of the second or the longitudinal furrow. After resting three to four hours more, the first line becomes visible again (fig. 24). It soon divides the egg into two equal halves as before. Immediately after (5—12 minutes) the second line of division makes its appearance at right angles to the first and divides the egg into four equal parts (fig. 26). This line generally makes its appearance first on one half close to the first furrow, and is soon followed by a corresponding line on the other half. They travel in opposite directions, until they meet with each other on the other side of the egg. Sometimes, as an exceptional case, two lines of the second furrow appear simultaneously on the two halves of the egg close to the first furrow at a distance of 90° from each other. These divide the egg into four equal parts as before, but only with this difference, that the four segments do not lie in the same plane. This irregularity is, however, soon lost in the next stage, when the division of the egg proceeds up to eight segments, the second vertical furrow appearing at the distance of 90° from the first vertical furrow. About two hours after the egg has been divided into four parts, the second line becomes narrower, and the four divided parts again coalesce into two. Some time later, the first transverse furrow becomes also fainter, and the egg apparently retrogrades to its first stage before segmentation, with the difference of having four nuclei instead of one (figs. 27 and 28). At the end of a short period of repose, the nuclei prepare to divide, and with this the original furrows are again restored in order of their appearance, soon followed, this time, by the second set of longitudinal furrows dividing the egg into eight equal parts (figs. 30, 31, 35, 36, and 37).

A similar case of segmentation is stated to occur in the egg of Lucifer by Brooks ($\frac{66-68}{14}$).

The segmentation to this stage may thus be compared to the first segmentation period of Mayer ($\frac{205-227}{3}$). After this it goes on regularly. Each of the eight spheres is now divided into two by the second set of equatorial furrows, and the egg therefore consists of sixteen equal spheres (figs. 32 and 38). In each of these the protoplasm takes the peripheral position and the deutoplasm the central. The nuclei of the egg can now be seen on the surface. After a pause a third set of longitudinal furrows appears almost simultaneously, and divides the egg into thirty-two parts (fig. 33). The central deutoplasmic portion of each segment now segments off from the peripheral protoplasmic and form "yolk-segments" (Balfour $\frac{168}{15}$) of unequal sizes (fig. 39, y.sg.), the cells arranged at the periphery being well marked from these segments. This seems to be an exception to what is generally seen among the Decapod eggs, where the apices of the segments, at such an early stage, are usually fused in the deutoplasmic mass in the centre of the egg, the mass showing no distinct divisions into segments. Transverse furrows now divide the egg into sixty-four parts. New yolk-masses are separated off from the segments. Longitudinal furrows again divide the egg into 128 parts (fig. 34). After the egg is divided into 256 parts by new furrows, its shape becomes spherical and the segmentation unequal.

Now the segments at a small area near one pole of the egg divide faster than the rest (figs. 41 and 42). This area is depressed a little, and the egg appears bean-shaped when viewed from the side. A section of this stage shows a row of lenticular cells near one pole (fig. 40, b l), separated from the yolk-mass, while the nuclei at the rest of the egg-periphery still occupy their position at the surface of the pyramidal cells.

Each "yolk-segment" (figs. 40, 43, 51, and 53) contains a number of yolk-spheres and clear vacuoles. The yolk-spheres are normally oval in shape, but become polygonal in hardened specimens. The vacuoles are of varying sizes, often very

small, but sometimes so large as to fill up nearly two thirds of
a yolk-segment. Within the yolk-segments are seen a fair
number of nuclei, placed not at the centre, but rather to one
side of them. Each nucleus is furnished with a dark-staining
nucleolus, and a layer of protoplasm prolonged into a reticu-
lum. I have not traced the origin of these nuclear bodies.
It is, however, probable that they are derived from the seg-
mentation nuclei. In one or two of my sections I have
observed them in eggs segmented to 128 parts. Balfour
figures and describes the nuclear bodies in the "yolk-seg-
ments" of Agelena, which in all particulars correspond with
what I have seen in those of Atyephira. Of the origin of the
nuclear bodies in the "yolk-segments," he says ($\frac{168}{15}$) :   "The
nuclei of the yolk-cells are probably derived from the nuclei
of the segmentation rosettes, and it is probable that they take
their origin at the time when the superficial layer of proto-
plasm separates from the yolk-columns below to form the blas-
toderm."

The depressed area appears white by reflected light.
Starting from this area, the lenticular cells are gradually
formed all over the rest of the surface, by the separation of the
superficial protoplasmic layer from the yolk-segments below.
While this is going on, the cells of the area become a little
thicker (fig. 43) and the depression smaller, and the egg again
assumes the elliptical form. The "yolk-segments" are now
of nearly equal size.

Gastrula, &c.—The white area, or "the Keimscheibe," is
depressed a little, and the egg appears bean-shaped from the
side (figs. 42 and 44). The cells near one point (nearer to one
pole of the egg) of this depression multiply faster than those
in other parts of the surface. These cells gradually sink down
into the yolk and eventually form a cup-shaped cavity whose
mouth is bounded by about twenty cells (figs. 45, 46, and 51,
g. m.). This cavity, or the gastrula, is at first very shallow,
but it soon grows inward and forward, so that it becomes com-
paratively deep (fig. 52, g. m.). While this is going on, an
elevation of the cells takes place on the middle part of the

depression, transverse to the long axis of the egg, and divides it
into an upper circular (figs. 45 and 46, *a.*) (containing the
gastrula cavity) and a lower oval one (figs. 45 and 46, *b.*).
The cells around the blastopore become much elongated, and
appear white by reflected light. The lower oval depression
disappears, while the blastopore and the circular depression
get much smaller (fig. 47). The white area around the blasto-
pore shifts upwards and takes a definite triangular shape (fig.
49, *a b*). The depression becomes shallow and the blastopore
closes (figs. 50 and 53, *g. m.*).

Germinal Layers.—With the formation of the gastrula,
we can already distinguish the origin of the germ-layers. The
endodermis formed from the invaginated cells of the gastrula,
while the rest of the blastoderm gives origin to the ectoderm.
The cells of the bottom of the cavity as well as those near the
blastopore gradually elongate, as was already said, and give off,
by continual division, the cells of the mesoderm (fig. 52, *m s*).
The formation of the mesodermic cells is more vigorous at
the forward edge of the cavity than on the floor, and the con-
sequence is that the opening of the cavity is gradually lessened,
as has been clearly shown by Reichenbach ($\frac{88}{16}$). At the time
of the closure of the gastrula, there is seen a mass of proto-
plasmic elements, aggregated just below the superficial ecto-
derm (fig. 53, *w. y.*), which is very likely to be compared to
the white yolk-elements of Reichenbach. After the closure of
the blastopore the endoderm cells gradually travel into the
yolk-segments, and their nuclei become indistinguishable from
those of the yolk (fig. 54, *h y*). Whether there exists a definite
cell-outline to each of the endoderm cells after they have
removed into the yolk, or whether the cell outline is lost, I
cannot tell with certainty.

At a region somewhat in front of the late blastopore, a fresh
invagination takes place, which gives rise to the permanent
anus (fig. 62, *pd.*). The triangular white patch becomes more
definite, and there is formed on each side of it, in front, a
circular elevation (figs. 55 and 56, *md.*), which later becomes
the mandible. The oval area in front of these becomes circu-

lar and forms the first rudiments of the carapace (figs. 54, 55, 56, *cp.*). On the side diametrically opposite to this two oval elevations (figs. 56, 58, 59, *oc.*) appear. These are at first somewhat separated from each other, but their interval is gradually lessened until they become connected together by an elevation of the intervening space. These are the first traces of the cephalic lobes. These gradually travel upwards (or morphologically backwards) as will be seen from figs. 60 and 61, *oc.* Immediately behind these the first traces of the first pair of antennæ become visible as oval elevations, and a little smaller than the cephalic lobes (fig. 60, *At.* 1). Then the second pair of antennæ (fig. 60, *At.* 2) is formed, so that the order of the formation of the parts of the embryo is as follows :—Abdomen, mandible, cephalic lobes, carapace, the first pair of antennæ, and the second pair of antennæ.

After a while the cephalic lobes come closer together, the first pair of antennæ elongates, and a crescent-shaped depression (fig. 61, *lb.*) is produced on the median line of the embryo in the region between the first pair of antennæ, which marks out the labrum. The second pair of antennæ, which up to this time was single, now becomes bilobed (fig. 61, *At.* 2), and the abdomen (fig. 61, *ab.*) takes a more spherical form. A section at this stage (fig. 62, *ab.*) shows that the cells of the abdominal region are much larger than those of other parts. The mesodermic cells (fig. 62, *ms.*) are very much crowded in the thoracic region, and a few in the cephalic and abdominal. This is the nauplius stage of the embryo.

In Palæmonites vulgaris, according to Faxon ($\frac{307}{17}$), " the parts of the embryo which first appear are the abdomen, the labrum, and the cephalic discs, and the first three pairs of appendages." The rudimentary carapace, which in Atyephira compressa appears before the first three pairs of appendages are formed, here comes into view after the third pair of maxillipedes is formed ($\frac{308}{17}$).

Stomodæum and Proctodæum.—Soon after the closure of the gastrula cavity a fresh invagination of the ectoderm takes place slightly in front of the late blastopore. This gives

rise to a very narrow tube—the proctodæum. The invagina-
tion deepens as the embryo grows larger, and the cells lining
its wall become columnar. The cells at the blind end of the
proctodæum later become continuous with the peripheral cells
of the yolk-mass (fig. 76), and thus the communication is
made between the proctodæum and the yolk-mass. Slightly
before the stage represented in fig. 61 a crescentic depression
is formed between the cephalic lobes and the first pair of
antennæ, which gives rise to the stomodæum. It is at first a
narrow blind tube like the proctodæum, and is directed upwards
and forwards; but as it grows it makes a sudden turn back-
wards, and its blind end considerably enlarges, forming a
spacious chamber, the future cardiac division of the stomach.
The communication between this chamber and the yolk-mass
is opened at a much later date than that of the proctodæum,
namely, at a stage slightly before the hatching of the embryo.
It will thus be seen that both of these invaginations arise
after the closure of the gastrula cavity, and independently
of it.

Secondary Mesoderm.—At the stage now described some
of the yolk-segments which lie close below the embryo become
markedly changed. They show a number of small granules
(fig. 62, sms.), which are easily coloured by logwood solution.
These granules are sometimes of considerable size, each having
a clear cellular outline. They gradually come out of the mass
and become transferred to take their position immediately
below the ectoderm (figs. 64 and 65, sms.), mingled with other
mesodermic cells. These are mostly aggregated in the cephalic
region, between the involutions of the ectoderm cells, but are
also found in all places. Their size is very small compared
with other cells, as will be seen in the figure. The time of
their appearance and their position seem to indicate that they
may probably be comparable to the " Secundäre Mesodermele-
mente" of Reichenbach ($\frac{149-152}{10}$), from which they differ,
however, in size and in general appearance.

Nervous System.—Although my observations on this head
are very imperfect, some of the sections I obtained show struc-

tures which appear to me of some interest. Up to the valuable contributions of Reichenbach almost nothing was known on the origin of the nervous system among the Decapod crustaceans.

The result of his investigation is briefly this ($\frac{152-159}{10}$). The whole nervous system arises from (1) the median groove, (2) the lateral strings, and (3) the depressions in the cephalic lobes. The cells of the lateral strings and the groove give rise to the ventral cord, while those of the cephalic depressions become the supra-œsophageal ganglion. Some of my sections of the nauplius seem to show the similar structure. Thus the fig. 63, which is the transverse section of an embryo through the cephalic region, shows the thickening of the ectoderm cells on both sides of the median line (fig. 63, *cd.*). In figs. 64 and 65, which represent two consecutive sections passing through the mouth opening, are shown the structures of the same nature. In fig. 66, a section cutting through the posterior part of the mandibles, the ectoderm is quite thick on both sides of the median line, which possibly corresponds to the lateral thickening of Reichenbach. Fig. 67, which represents a section similar to fig. 66, but of an embryo slightly older than it, shows two circular masses of cells on each side of the median line below the ectoderm cells. These appear to be the section of the two lateral portions of the ventral nerve-cord after its separation from the superficial ectoderm. But as these are the only sections by which I can get any knowledge of the origin of the nervous system, and as I have neither traced the origin nor the fate of the structures described, I have not written here anything more than a short description.

The embryo gradually gets larger (fig. 68). New appendages (fig. 68, *mx.* 1, 2, and *mxp.* 1) are formed, behind the mandibles, in regular succession. The two maxillæ (fig. 68, *mx.* 1, 2) are at first single appendages like the mandible (*md.*), but soon become bilobed. The appendages behind the maxillæ are bilobed from the time of their appearance. The appearance of the maxillæ as single-lobed appendages differs a little from the case of Palæmonites ($\frac{308}{17}$), where they are bilobed from

the start.   From the embryo of P a n o p e u s ($\frac{1}{18}$) it differs in
the fact that the second pair of maxillæ of that Crab is bilobed
from the beginning, and is not single as in Atyephira.

At the base of the second pair of antennæ is now seen the
first trace of the antennal gland (fig. 68, *gg.*, and fig. 90).   The
ectoderm cells group themselves at this spot into a circular
mass, the cells of which are well distinguishable from other
cells by their regular roundish shape.

The superficial ectodermic cells of the cephalic lobes (fig. 68,
oc.) become marked off from the inner layers.   The labrum
(fig. 68, *lb.*) pushes downwards, so as to lie between the second
pair of antennæ (fig. 68, *At.* 2).   Beneath the labrum is seen
the œsophagus (fig. 68, *œ.*) as a square opening.   New thoracic
segments become visible behind the first pair of maxillipedes.
The abdomen gets larger, and its end becomes bilobed.   In the
notch between the two lobes is the anus (fig. 68, *an.*), bounded
by about thirteen or fourteen cells.   Continued from the anus
is seen the latter part of the intestinal canal, lined with
columnar epithelium.   No nauplius eye has as yet appeared.

In fig. 69 all the pairs of the maxillipedes (*mxp.* 1, 2, and 3)
have appeared.   Both antennæ (*At.* 1 and 2) have changed
considerably.   The cephalic lobes become more definite in out-
line.   The cells of the superficial ectoderm, which later become
the crystalline cones, elongate.   The abdomen gets still longer,
five succeeding segments behind the third pair of maxillipedes
have appeared, and the future telson is marked off from the
abdomen at the sides.   The outline of the carapace is now
seen extending to over the third pair of maxillipedes.   The
depression of the antennal gland becomes deeper.

Fine nervous striations now become visible within the
supra-œsophageal cellular mass (fig. 69, *sgn.*), whence the
branches are given out first to each of the cephalic lobes.
Each of these branches sends out a branchlet near its base to
the median ocellus (*ocl.*), the nauplius eye, which is now
formed.   Behind they run downwards and surround the
œsophagus, giving off branches to both antennæ.

Traces of the nervous striæ are also seen faintly on each

side of the median line as far back as to the segment bearing
the first pair of maxillæ.   These give out short branches to
the mandibles.

At a little later stage than the last, a short spine-like process
(fig. 70, *d.s.*) becomes visible on the dorsal median line of the
carapace.   This is the rudimentary dorsal spine, which so com-
monly occurs in the Crab Zoea.   At the posterior end of the
yolk-mass, where it joins the proctodæum, small vacuoles and
oil drops (figs. 70 and 71, *lv.*), both of very refractive appear-
ance, appear.   I have not clearly followed the development of
these vacuoles and oil drops, but I am inclined to consider them
as the first indication of the liver.

The heart (figs. 70, 71, *h.*) now appears on the dorsal aspect
of the embryo within the mesodermic cells occupying the
position just outside the place where the liver (?) globules have
appeared.   I have not obtained any good section which shows
the origin of the heart, but my observations tend to show that
it is mesodermic in its origin.   The pigment of the eye is now
seen for the first time in this stage.

More segments come into view, and at the stage represented
in figs. 72 and 73, the segments posterior to the last pair of
maxillipedes have increased up to ten.

The first pair of antennæ (*At.* 1) now shows a slight con-
striction on its sides, thus marking out the future basal and
the proximal portions.

The second pair of antennæ (*At.* 2) which is as long as the
first pair, shows the future flagellum and the scale, the latter
considerably broader than the former, and beset with a number
of short setæ on its upper side.   The flagellum is bifurcated
at its tip.

The mandible (figs. 72, 73, *md.*) shows no definite structure
as yet.

The first and the second pairs of maxillæ and the first pair
of maxillipedes (figs. 72 and 73, *mx.* 1, 2, *mxp.* 1) have consi-
derably changed.   They all show traces of the future lobules on
their inner sides.   The exopodite of the first pair of maxillæ is
furnished with three short points, while those of the second

pair of maxillæ and the first pair of maxillipedes are provided with two such.

The second and the third pairs of maxillipedes (figs. 72, 73, *mxp.* 2, 3) show no marked change except in size. The telson becomes more definite in outline. It is somewhat notched on its inner angles. Inside these notches, on the posterior border of the telson, are seen five rudimentary setæ.

The nervous striations have increased very much. Those going to the cephalic lobes expand into the shape of a fan in the anterior third of the lobe. The ocular pigments have also considerably increased. The ocellus has grown larger.

The yolk-segments are now seen to have a radial arrangement with their nuclei on the periphery. These segments fuse together in the centre.

Within the intestinal canal (fig. 75, *in.*) are already seen the concretions of extraneous matter.

A longitudinal section through this stage (fig. 76), shows that the epithelial cells have been considerably formed at the periphery of the yolk-mass near the anterior end of the future hind gut (in fig. 76, just below the heart, *h.*). Five bundles of flexor muscles (*f.m.*) are also seen in the abdominal region. Each of these consists of an aggregation of spindle-shaped cells, with oval nuclei. No striations have yet made their appearance within the cells.

The yolk-mass, which up to this time has been uniformly oval, now changes its form. A large space (figs. 77, 78) is formed in front of the cephalic lobes by the absorption of the yolk there. On each side of the embryo, about in the line with the second pair of maxillipedes, a constriction occurs. A similar constriction takes place on each side of the cephalic lobes. Thus the yolk-mass shows five lobes, one antero-median, two lateral, and two posterior. The lateral and the posterior lobes later differentiate into the liver.

About two days later the embryo presents the following characters.

The anterior-median lobe of the mesenteron, which is seen in the last figure as a single lobe, becomes elevated from the

rest, and shows two lobes plainly. These lobes indicate the future cæcal ends, (fig. 79, *ce.*) situated above the pyloric division of the alimentary canal.

The first pair of antennæ (fig. 80, *At.* 1.) becomes two-jointed, bearing four setæ at its extremity. The endopodite has not yet appeared.

In the second pair of antennæ (fig. 80, *At.* 2), the setæ of the exopodite (*At.* 2, *ex.*) have much increased both in size and number. From the internal branch two long setæ become visible.

The mandible is now a bilobed appendage ; the anterior lobe, which is larger, shows two rows of about seven teeth. A small moveable appendage (endopodite ?), beset with minute setæ on its border, is seen at the end of this lobe.

Both lobes of the first pair of maxillæ (fig. 81) have a number of setæ on the ends. The inner side of the inner lobe shows about five lobules beset with setæ.

The second pair of maxillæ (fig. 82) is little smaller than the first pair, and is furnished with numerous setæ on its inner lobe, which is cut into five lobules.

The first, second, and the third pairs of maxillipedes (fig. 83) have not undergone much change. They are, however, all provided with setæ on their extremities.

Behind the maxillipedes the first pair of ambulatory legs (fig. 83, *amb.* 1) becomes visible as a simple bilobed appendage.

The dorsal spine is lost.

About twenty-four hours afterwards the mandible and the maxillæ (fig. 84, *md.*, *mx.* 1, 2) have undergone great changes. The scaphognathite (fig. 84, *mx.* 2, *s. g*) of the second pair of maxillæ is seen in rapid motion. The pigment patches now appear at different parts of the body. Their distribution is almost similar to the first stage after hatching.

Embryo just hatched (fig. 85).—About twelve hours after the stage last described the embryo is hatched. It measures about $3\frac{1}{4}$ mm. in length. The carapace is broad, produced between the eye into a rostrum (*rs.*), at the base of which is a simple median eye (*ocl.*). The compound eyes (*oc.*) are large,

supported upon very short stalks. The abdomen consists of but six segments, the telson being still united with the last. The broad triangular fin (fig. 86) is furnished with fourteen long setæ, each of which is finely feathered on both sides, except the two outermost pairs, which are feathered only on the inner side. The three inner pairs of the long setæ are, moreover, provided with short spines on both sides. The spaces between the long setæ are furnished with short setæ $(\frac{310}{17})$. Within the abdominal segments, from the first to the fifth, are seen ganglia (fig. 85, *n.*) of the ventral nerve-cord, united by double commissures. The first, second, and the third ganglia are large, and spherical in form, while the two succeeding ones are small. The anus is seen as a longitudinal slit on the lower side of the telson.

The first pair of antennæ (fig. 85, *At.* 1) consists of a basal segment, and a short distal one, which carries four setæ, two of which are the modified sensory organs. The outer two are of unequal length, the shorter being feathered on both sides. The proximal segment carries a single, long, large seta on its distal end.

The second pair of antennæ (fig. 85, *At.* 2) is nearly of the same length as the first pair. It consists of a short distal segment, with two proximal branches, the outer of which (the future flagellum) is large, and furnished with thirteen setæ on its distal margin. The inner branch is slender, and furnished with two setæ of unequal length, the longer of which is feathered on both sides. The shorter is curved, and bears a small, bud-like appendage on its inner side near the base. There is also a short, stout spine at the base of the inner branch on the distal end of the proximal segment of it.

The green gland (fig. 85, *gg.*, figs. 93 and 94) is situated at the base of the proximal segment, its opening being perched on a little eminence on the inner side of the segment.

The labrum (fig. 85, *lb.*) is large, lying slightly posterior to the second pair of antennæ.

The mandible (fig. 84, *md.*) consists of two branches, the anterior of which is furnished with numerous teeth, and the

posterior branch is divided into two points.   A small, single-jointed appendage, feathered on both sides, is seen on the proximal end of the anterior branch as in the preceding stage. This appendage is lost in future stages.

The first pair of maxillæ (fig. 87) consists of a long piece with two lobes, the outer, or basipodite (fig. 87, *bp.*), bearing three setæ, and the inner, or coxopodite (*cxp.*) with four setæ. On the outer border of it, somewhat proximal to the division of the lobes, a single-jointed palpus, or endopodite (fig. 87, *en.*), with two long feathered setæ, is placed.

The second pair of maxillæ (fig. 88) is about as long as the first, but is much the broader.   It is divided into an inner and an outer lobe.   The former consists of a large endopodite (*en.*), beset with six rather short setæ on its inner border; a small basipodite (*bp.*) consisting of two lobes, both of which bear long setæ, and a coxopodite (*cxp.*), also divided into two lobes at its extremity, bearing setæ.   The outer branch of the second pair of maxillæ is a large scaphagnathite (*sg.*) beset with setæ.

The first pair of maxillipedes (fig. 89) consists of a broad basal segment, and two terminal ones.   The outer branch (*ex.*) is considerably longer than the inner, and consists of two joints, bearing five long setæ on its extremity.   The inner branch (*en.*) is about two thirds the length of the outer.   It consists of four joints, beset with a few setæ on its inner border.   The basal segment also bears short setæ on its inner border.

The second and the third pairs of maxillipedes are similar in structure to the first pair, except that the basal segment of the second pair of maxillipedes is less broad than that of the first pair, and that of the third is again less than that of the second, while the length of the entire appendage increases in the reverse order.

Thoracic Appendages.—Four pairs of the rudiments of these appendages (fig. 85, *amb.* 1—4) are already formed, each presenting two lobes.   The first pair is about half as long as the third pair of maxillipedes.   Each of the two branches of the

first and the second pairs and the inner branch of the two last terminate in two rudimentary spines.

Within the branchiostegite two rudimentary gills are seen above the first and the second thoracic legs. The anterior is about three times as large as the posterior, and shows four simple lobes within it, while the posterior is as yet a simple sac. Blood-corpuscles are seen in rapid motion inside these gills.

No trace of the abdominal appendages is as yet visible.

Pigments.—Two large blotches of pigments are seen just behind the eye-stalks. Three smaller ones are also seen above the branchial chamber on each side of the carapace, and one on the median line of the abdomen. On the sternal aspect there is a large patch at the foot of the rostrum just above the ocellus, and a series of large median pigments are seen on the posterior part of each of the abdominal segments. On the last segment three patches are distributed, one above the anal opening and the two others on the two lobes of the fin.

The Antennal Gland.—The first trace of this gland (figs. 68 and 90, *gg.*) becomes visible at the base of the second pair of antennæ at the time when the first pair of maxillipedes appear in the embryo. The cells which are concerned in the formation of it are all ectodermic, and are at first about eight in number (fig. 90). They form a circular group near the inner side of the antenna. The median part of this is depressed, and forms a shallow cup-shaped cavity with a tolerably large mouth (fig. 91). The protoplasm of the cells that are thus depressed show granulations by which the area can be easily detected in the surface view. The depression becomes gradually deeper (fig. 92), while its opening gets smaller and becomes produced outward. The involution goes on still further. Granular fluid is already formed in the cavity, sometimes before the hatching of the embryo. The canal formed by the involution becomes twisted round among the mesodermic cells, and by the time the embryo is hatched its convolutions are about three or four in number (figs. 93 and 94).

Olfactory Setæ.—At the stage of the embryo represented in fig. 79 the first trace of the olfactory setæ becomes visible.

The extremity of the first pair of antennæ (fig. 80, *At.* 1, and fig. 95) is at this time furnished with four setæ. At the base of each of the two inner setæ there is a nucleus enclosing a darkly-staining nucleolus. No definite external distinction can as yet be made between these and other setæ. Gradually, however, these become distinct from others (fig. 96). Granulations appear on the upper part of the two setæ destined to become the olfactory organ. A constriction occurs at the base of these setæ, where the nerve-fibre, running from the ganglionic mass, ends in an enlargement. At the time the embryo is hatched they show the appearance shown in fig. 96. After some time, when the embryo is about 4 mm. in length, they become spatula-shaped, their ends present the form of a prominent papilla (fig. 97).

At each moulting they appear in twos on the distal end of each segment, in the same manner as the two first were formed.

The further changes of the embryo correspond in the main with what has been observed by Faxon on Palæmonites ($\frac{3\,0\,3\,-\,3\,2\,3}{1\,7}$). After each moulting the embryo gets more of the characters of the adult. The last pair of abdominal appendages become visible as two oval plates within the caudal fin soon after the first moulting. The other appendages are regularly formed from behind forward, while the number of the ambulatory legs becomes complete.

In the embryo measuring 4 mm. (i. e. two moults after the hatching of the embryo) the caudal fin or telson separates from the sixth abdominal segment.

Branchiæ other than those already mentioned are regularly formed. A rudiment of the podobranchiæ is first seen at the coxopodite of the second pair of ambulatory legs as a simple sac, similar to those of the pleurobranchiæ. These (podobranchiæ) next appear almost simultaneously on the other ambulatory legs excepting the last, and then successively on the third, second, and first maxillipedes. Of these only one pair (i. e. that on the second pair of maxillipedes) develops into a permanent gill, while five others (those on the third pair of

maxillipedes, and the first, second, third, and fourth pairs of ambulatory legs) modify into peculiarly-shaped appendages, the tips of which are provided with a structure like avicularia (fig. 98, *pdb'*.), while the one other (i. e. that on the fourth pair of maxillipedes) remains as a simple sac.

While these branchiæ are being formed a long seta develops on the coxopodite of each of the ambulatory legs, just in front of them. These setæ are at first comparatively very long. Their number gradually increases while their size diminishes, until in the adult prawn we have a dozen setæ of moderate length (fig. 98, *cxs*.).

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EXPLANATION OF PLATES XXV, XXVI, XXVII, AND XXVIII,

Illustrating Mr. Ishikawa's Paper "On the Development of a Freshwater Macrurous Crustacean, Atyephira compressa, De Haan."

List of Reference Letters.

amb. Ambulatory leg.   *an.* Anus.   *At.* 1. Autenna.   *At.* 2. Antennule. *b. c.* Blood-corpuscles.   *bl.* Blastoderm.   *b. s.* Blood space.   *bp.* Basipodite. *c. d.* Cephalic depression.   *ce.* Cæcum.   *cp.* Carapace.   *cxp.* Coxopodite. *cx. s.* Coxopoditic setæ.   *c. t.* Connective tissue.   *d. s.* Dorsal spine.   *e. e.* External epithelium.   *en.* Endopodite.   *ep.* Ectoderm.   *f. e.* Follicular epithelium.   *f. m.* Flexor muscle.   *ger.* Germogen.   *g. g.* Green gland.   *g. m.* Gastrula mouth.   *h.* Heart.   *hy.* Endoderm.   *i. e. o.* Internal epithelium of the ovary.   *i. e. od.* Internal epithelium of the oviduct.   *in.* Intestine.   *lb.* Labrum.   *lv.* Liver.   *mb.* Structureless membrane.   *md.* Mandible.   *mx.* Maxilla.   *mxp.* Maxillipede.   *ms.* Mesoderm.   *n.* Nerve.   *n. c.* Nucleus of the connective tissue.   *o. od.* External orifice of the oviduct.   *od.* Oviduct. *ocl.* Simple eye.   *OC.* Compound eye.   *ol. s.* Olfactory setæ.   *œ.* Œsophagus. *p.?* Palp ?   *pd.* Proctodæum.   *p. o.* Primordial ova.   *pdb'.* Podobranchia modified.   *rs.* Rostrum.   *sd.* Stomodæum.   *sg.* Scaphognathite.   *s. gn.* Supraœsophageal nerve ganglion.   *s. ms.* Secondary mesoderm.   *vit.* Vitellogen. *vac.* Vacuole.   *w. y.* White yolk.   *y. s.* Yolk-spherule.   *y. sg.* Yolk-segment.

FIG. 1.—Coxopodite of the third ambulatory leg of the female Atyephira, showing the external orifice of the oviduct.   × 12.

Fɪɢ. 2.—A full-grown ovary viewed from ventral side, showing the germinal band. × 20.

Fɪɢ. 3.—A transverse section of a middle-sized ovary. Drawn with camera A and 2 (Carl Zeiss).

Fɪɢ. 4.—Connective-tissue layer of the ovary, treated with acetic acid. Camera ᴅ ᴅ and 4.

Fɪɢ. 5.—External epithelium layer of the ovary. Camera ᴇ and 2.

Fɪɢ. 6.—External epithelium layer of the oviduct. Camera ᴇ and 2.

Fɪɢ. 7.—A portion of the wall of the vitellogen viewed from inside, showing a small capillary branch. Camera ᴅ ᴅ and 4.

Fɪɢ. 8.—A transverse section of the ovary wall, showing the blood space. Camera ᴅ ᴅ and 4.

Fɪɢ. 9.—A transverse section of a young ovary of about 0·14 mm. in cross diameter. Camera ᴅ ᴅ and 4.

Fɪɢs. 10 and 11.—Transverse sections of a larger ovary, passing through the oviduct. Camera ᴅ ᴅ and 4.

Fɪɢ. 12.—Three young eggs from the germogen, showing the growth of germinal dots. Camera ᴅ ᴅ and 2.

Fɪɢ. 13.—A section of a young ovum with developing yolk-spherules. Camera ᴅ ᴅ and 2.

Fɪɢ. 14.—A section of a germinal vesicle, surrounded by a network of protoplasm. Camera ᴅ ᴅ and 2.

Fɪɢ. 15.—Internal epithelium of the oviduct during the breeding season. Camera ᴅ ᴅ and 4.

Fɪɢ. 16.—Primary egg-membrane, showing the polygonal markings. Camera ᴅ ᴅ and 4.

Fɪɢs. 17—40.—Magnified 40 diameters.

Figs. 17, 18, and 19.—Eggs dividing into two equal parts.

Fig. 20.—First transverse furrow completed.

Figs. 21, 22, and 23.—First resting stage.

Fig. 24.—First transverse furrow restored.

Fig. 25.—First longitudinal furrow appearing.

Fig. 26.—First longitudinal furrow completed. The egg is divided into four equal parts.

Figs. 27 and 28.—Second resting stage.

Fig. 29.—First transverse furrow restored.

Fig. 30.—First longitudinal furrow restored.

Fig. 31.—Egg divided into eight equal parts by the second longitudinal furrow.

Fig. 32.—Egg divided into sixteen equal parts by the second set of transverse furrows.

Fig. 33.—Egg divided into thirty-two parts by the third set of longitudinal furrows.

Fig. 34.—Egg divided into sixty-four parts by the third set of transverse furrows.

Fig. 35.—Egg at the end of the second resting stage, seen by the transmitted light.   A weak acetic acid preparation.

Figs. 36 and 37.—Two longitudinal sections of an egg of the same stage as Fig. 30.

Fig. 38.—A longitudinal section of an egg divided into sixteen equal parts.

Fig. 39.—A longitudinal section of an egg divided into sixty-four parts.

Fig. 40.—A longitudinal section of an egg divided into 256 parts.

Figs. 41 and 42.—Two views of an egg, showing the germinal disk.   Immersed in Kleinenberg's picro-sulphuric acid for about fifteen minutes.   × 65.

Fig. 43.—A longitudinal section of the germ disk of an egg of the same stage as Figs. 41 and 42.   Camera B B and 2.

Fig. 44.—Side view of an egg of the stage slightly later than that represented by Fig. 42.   × 40.

Figs. 45, 46, 47, and 48.—Different stages of the gastrula of the egg. Front and side views.   × 40.

Figs. 49 and 50.—Gastrula cavity nearly closing.   × 40.

Fig. 51.—A longitudinal section through the gastrula cavity of an egg at the same stage as Figs. 45 and 46.   Camera B B and 4.

Fig. 52.—A longitudinal section through the gastrula cavity of the stage represented in Figs. 47 and 48, showing the formation of mesoderm cells. Camera D D and 2.

Fig. 53.—A longitudinal section through the gastrula cavity at the stage represented in Figs. 49 and 50, showing the closure of the blastopore. Camera B B and 4.

Fig. 54.—A longitudinal section through the region where the gastrula cavity has closed.   Camera D D and 2.

Figs. 55 and 56.—Two views of an embryo in which the carapace (*cp.*), mandibles (*md.*), and the cephalic lobes (*oc.*) have appeared.   × 40.

Figs. 57, 58, and 59.—Three views of an embryo, slightly more developed than the last.   × 40.

Fig. 60.—Embryo still more developed, seen from embryonic pole.   × 40.

Fig. 61.—Nauplius stage of an embryo, viewed from embryonic pole.   × 40.

Fig. 62.—A longitudinal section of an embryo represented by Fig. 61. Camera D D and 2.

Figs. 63, 64, 65, and 66.—Three consecutive transverse sections of the nauplius, showing the formation of the nervous system.   Camera D D and 2.

Fig. 67.—A transverse section of a nauplius, slightly older than the last. Camera D D and 2.

Fig. 68.—Surface view of an embryo older than Fig. 61.   × 150.

Fig. 69.—Embryo older than the last. Simple median eye and nerve striations are for the first time visible. × 150.

Figs. 70 and 71.—Embryo slightly older than Fig. 69, showing the formation of liver globules. × 40.

Figs. 72 and 73.—Two stages of an embryo slightly older than that represented in Figs. 70 and 71. × 150.

Figs. 74 and 75.—Two views of an embryo of the same stage as Fig. 73.

Fig. 76.—A longitudinal section of an embryo of the stage represented by the Figs. 74 and 75. × 65.

Figs. 77 and 78.—Embryo further developed than the last, showing a change occurring in the form of the yolk-mass. × 40.

Fig. 79.—Embryo about two days after the last, showing the intestinal cæca. × 40.

Fig. 80.—Two pairs of antennæ of the embryo given by Fig. 79. × 150.

Fig. 81.—First maxilla of the same. × 150.

Fig. 82.—Second maxilla of the same. × 150.

Fig. 83.—Three maxillipedes and the first ambulatory leg of the right side of the same embryo. × 150.

Fig. 84.—A labrum, a mandible, and maxillæ of an embryo about twenty-four hours older than the one represented by Fig. 79. × 150.

Fig. 85.—Embryo just hatched, from below. × 30.

Fig. 86.—Telson of the same. × 60.

Fig. 87.—First maxilla of the same. Camera D D and 4.

Fig. 88.—Second maxilla of the same. × 150.

Fig. 89.—First maxillipede of the same. × 150.

Fig. 90.—Surface view of an antennal gland of an embryo represented in Fig. 68. × 400.

Fig. 91.—Antennal gland of an embryo slightly more developed than Fig. 68. × 400.

Fig. 92.—Antennal gland of an embryo of about twelve hours before hatching. × 400.

Fig. 93.—Antennal gland of an embryo just hatched. × 400.

Fig. 94.—A section of the same. × 400.

Fig. 95.—Extremity of the first antenna of the left side, from an embryo represented by Fig. 79, showing the olfactory setæ. × 400.

Fig. 96.—The same of the right side, from an embryo just hatched. × 400.

Fig. 97. – The same from an embryo 4 mm. in length. × 400.

Fig. 98.—First ambulatory leg of the left side of an adult animal. × 10.

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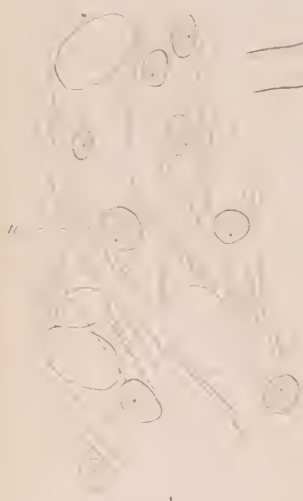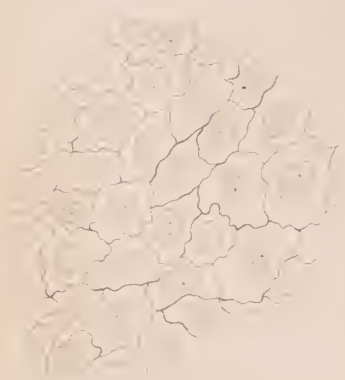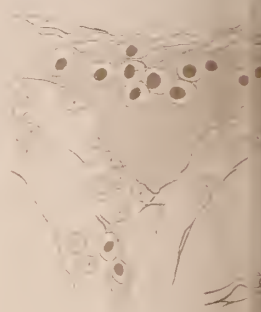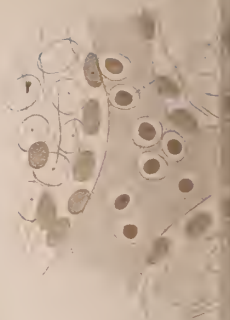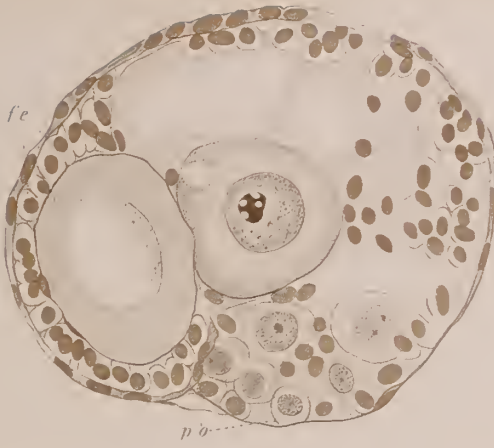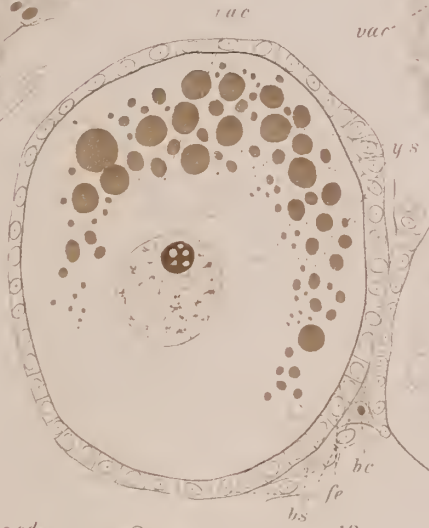
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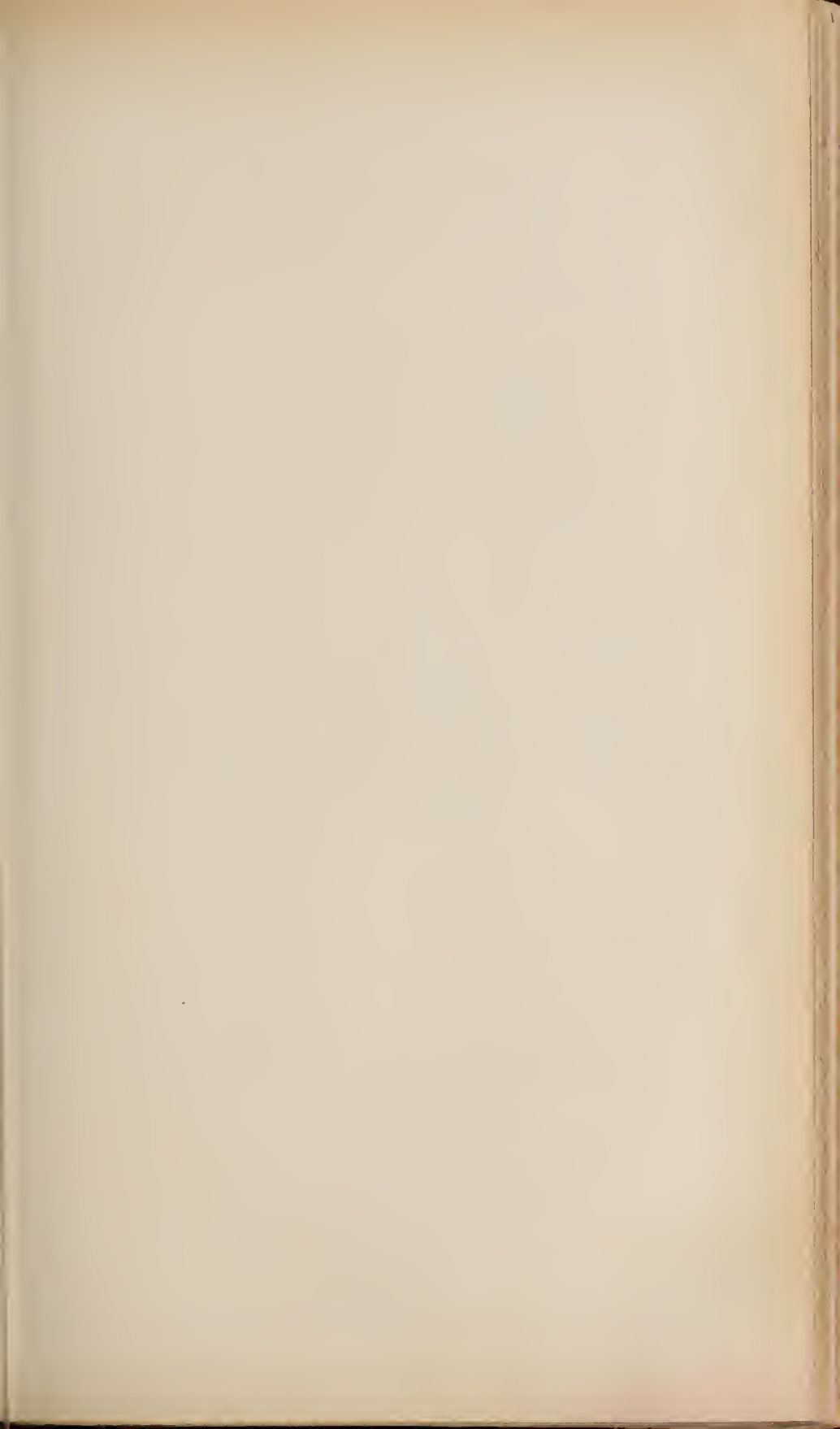
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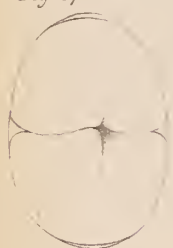
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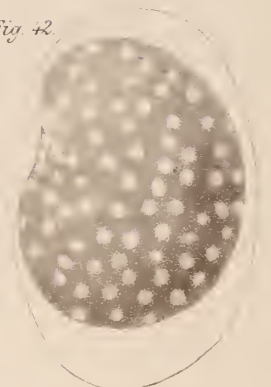
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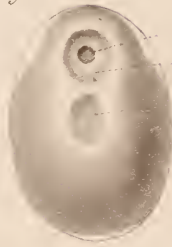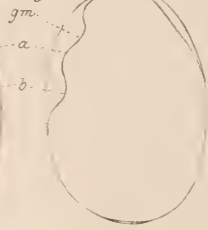
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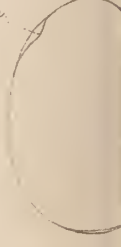
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Fig. 23. Fig. 24 Fig. 25. Fig. 26

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Fig. 39. Fig. 40. Fig. 43. Fig. 51. Fig. 53.

Fig. 49. Fig. 50 Fig. 52. Fig. 54.

Fig. 60 Fig. 61.

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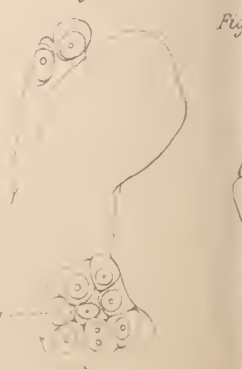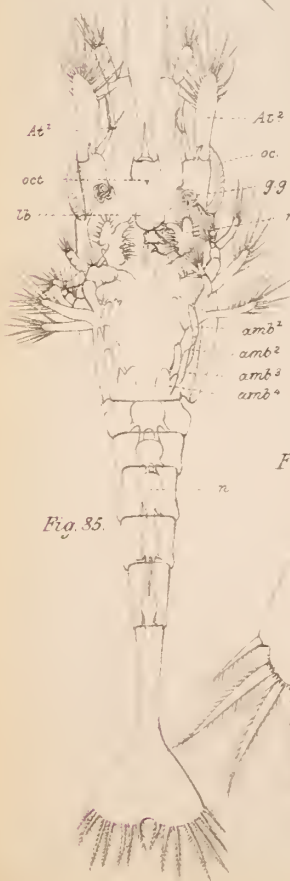
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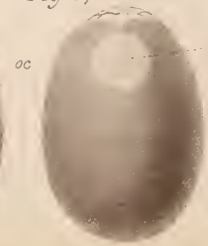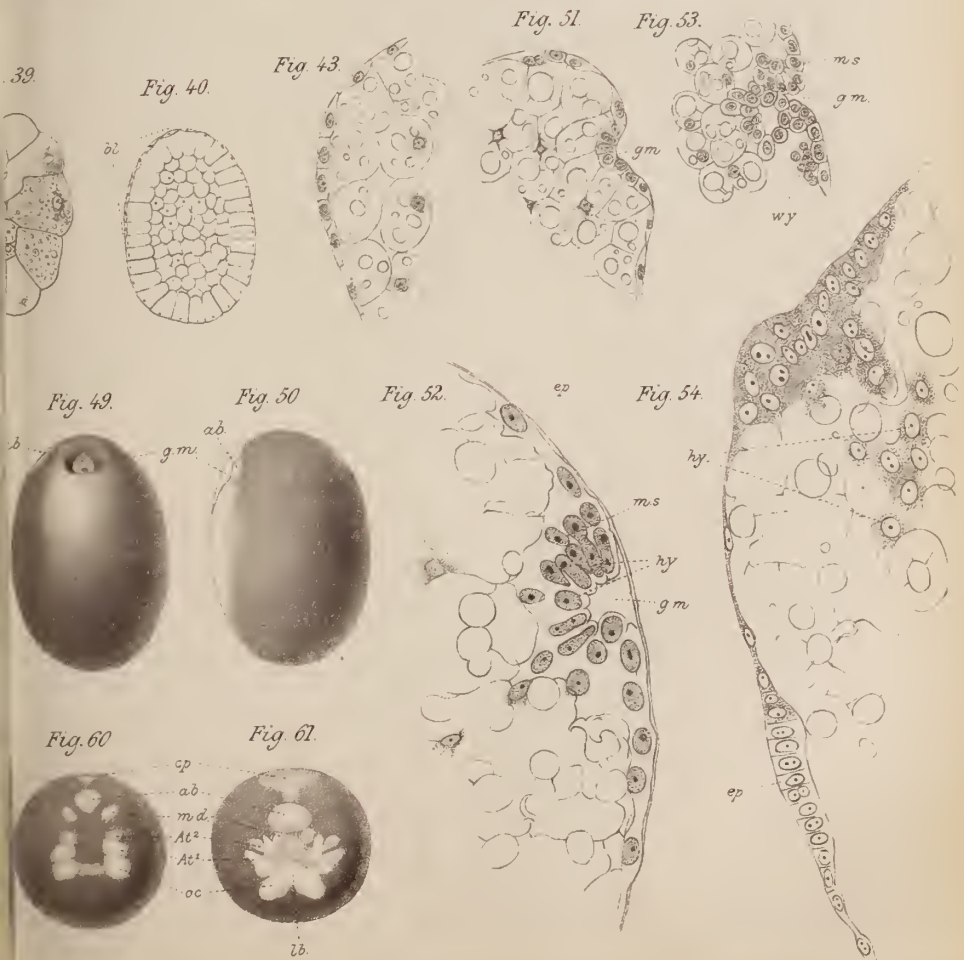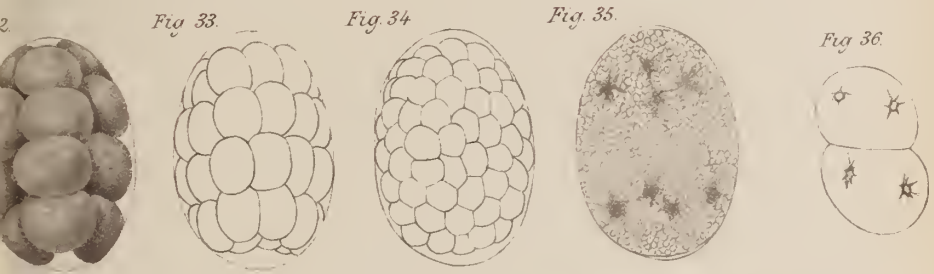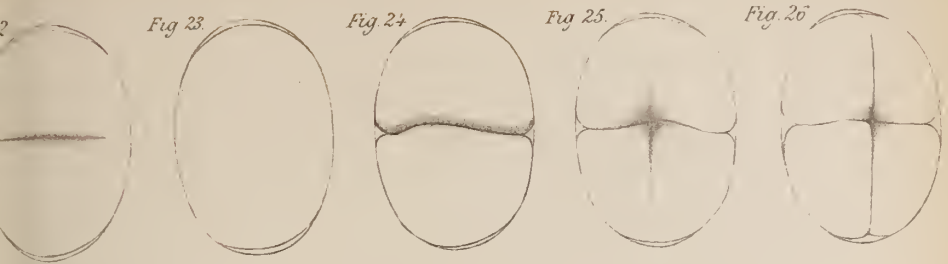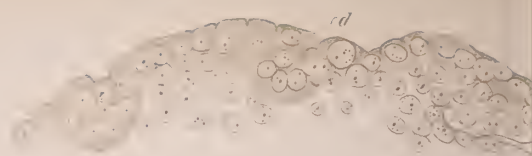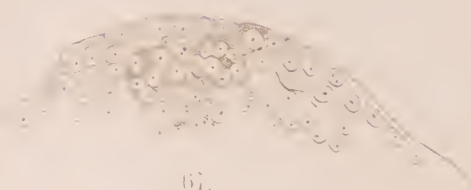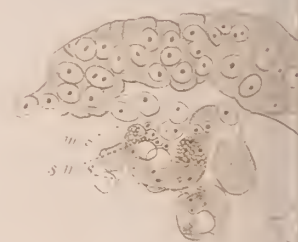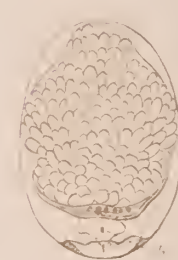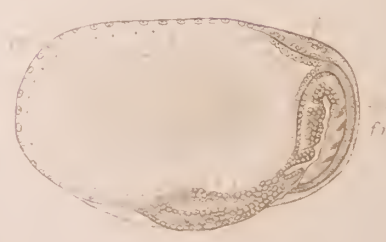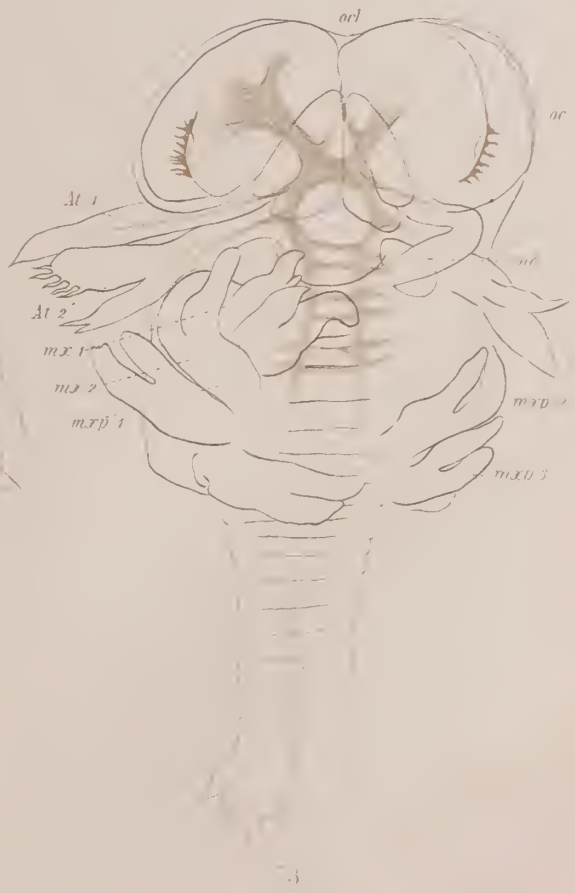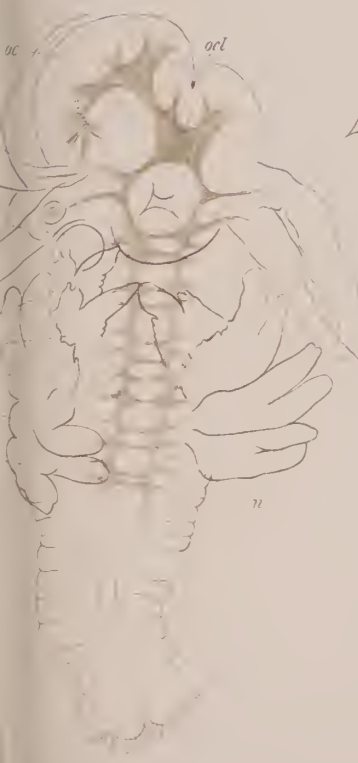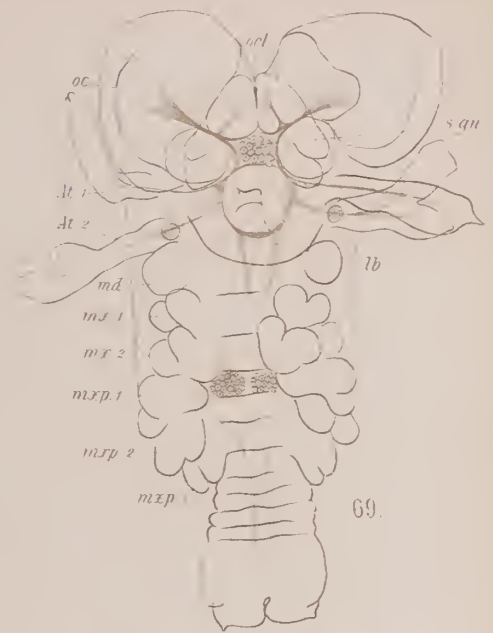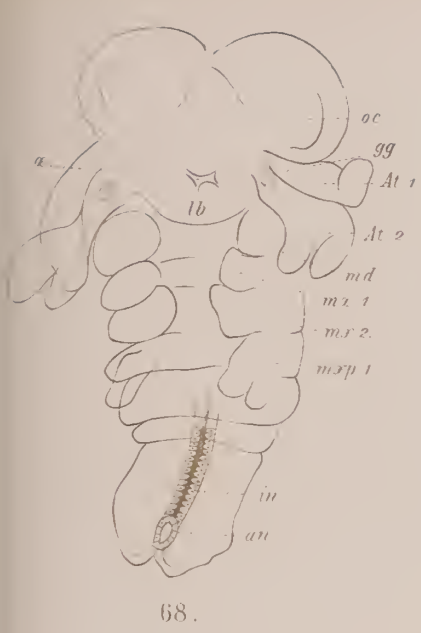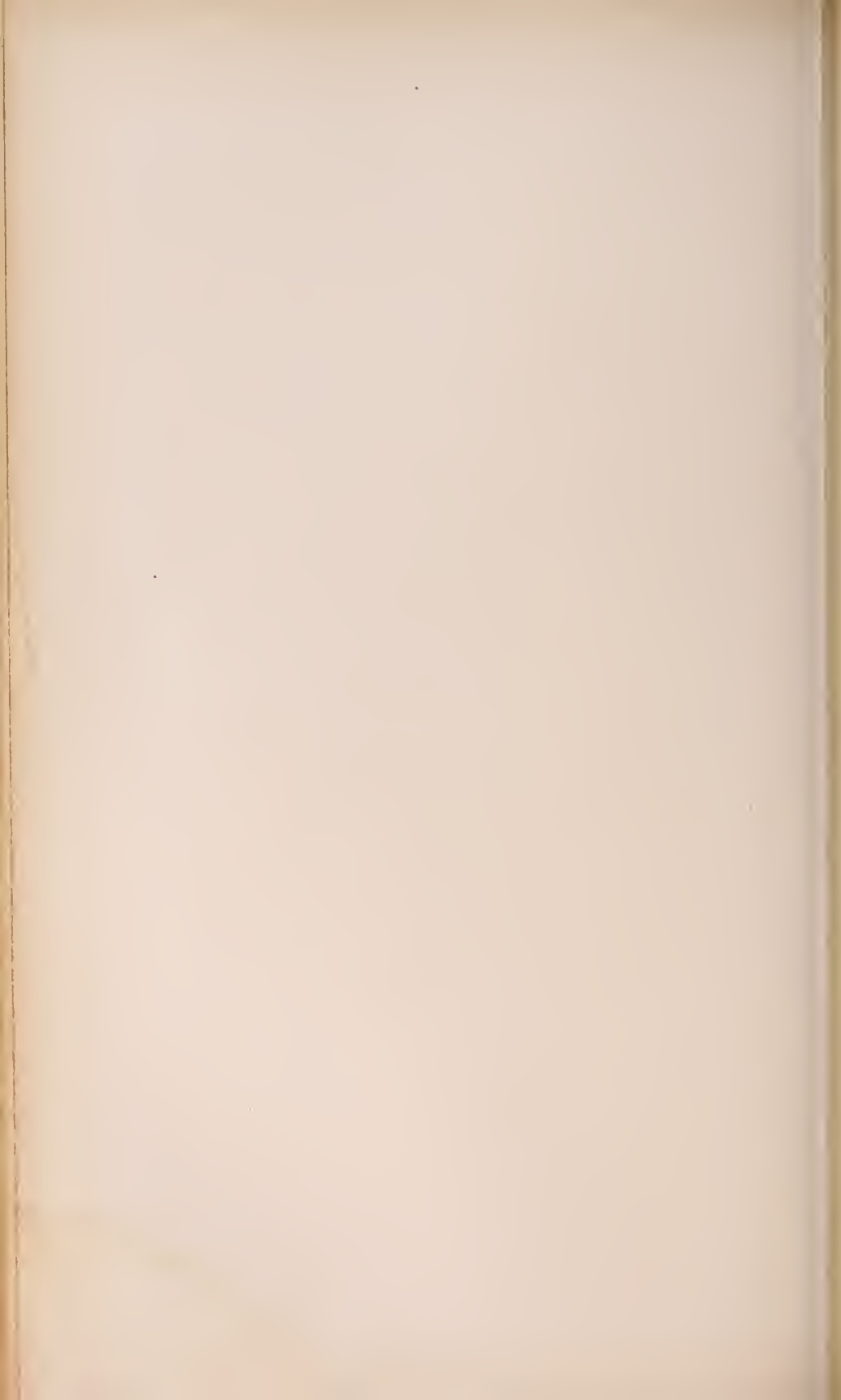
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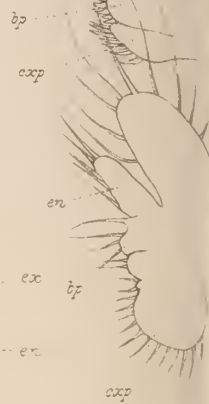
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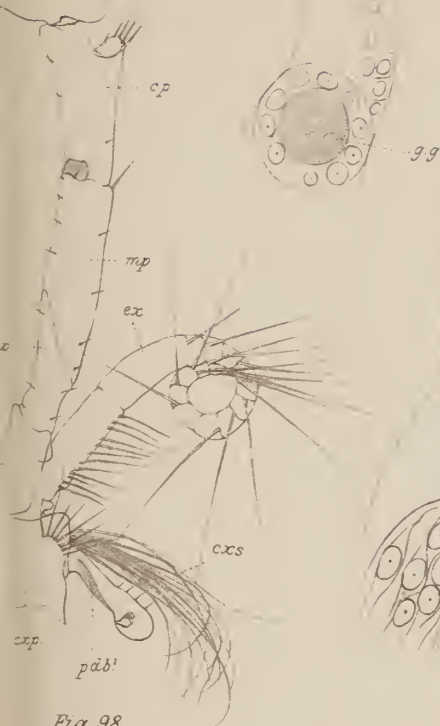Fig. 85   Fig. 89   Fig. 90

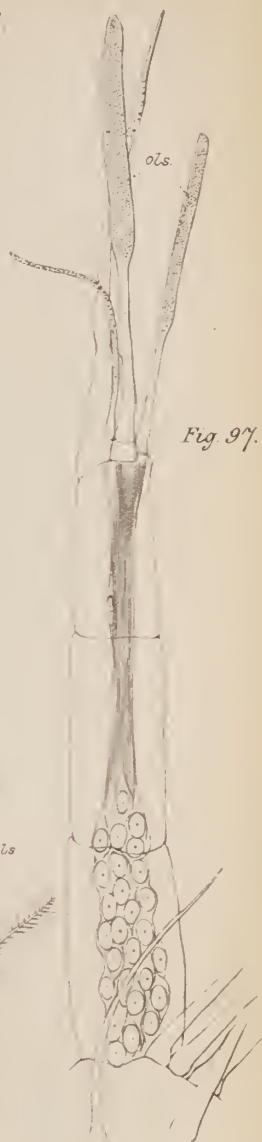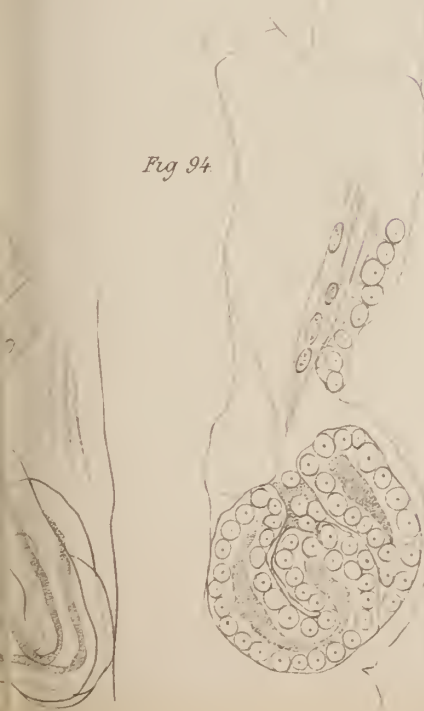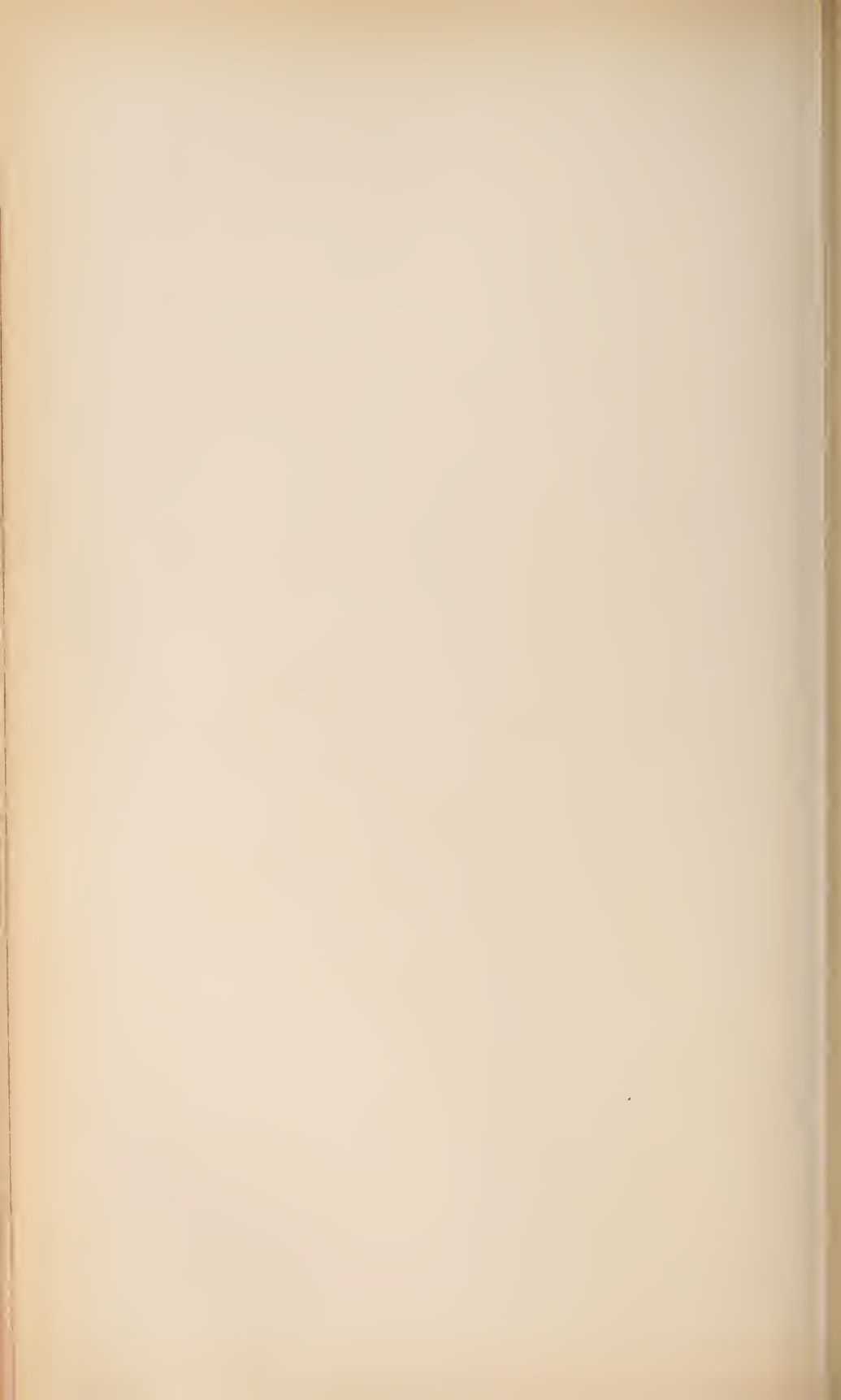Fig. 91

Fig. 95

Fig. 97.

Fig. 98.

Fig. 94

Fig. 96

Fig. 92.

On the Supposed Communication of the Vascular System with the Exterior in Pleurobranchus.

By

Alfred Gibbs Bourne, D.Sc. Lond., F.L.S.,
Assistant Professor of Zoology and Comparative Anatomy in University College, London.

With Plate XXIX.

LACAZE-DUTHIERS[1] has described in Pleurobranchus a special canal opening on the one hand to the exterior and on the other to the branchial vein, and in Dentalium, two orifices leading from the exterior into the two great veins in the mantle.

These statements have remained hitherto unchallenged. I have not examined Dentalium in this respect, but in the case of Pleurobranchus I am in a position to deny the existence of any such communication.

At the request of Professor Lankester, who had long desired to re-examine the structure described by Lacaze-Duthiers in Pleurobranchus, I prepared some specimens of this mollusc when at Naples in the spring of last year, and I have recently, at Professor Lankester's request, completed the examination of this material in the laboratory of University College.

The orifice described by Lacaze-Duthiers in Pleurobranchus may be easily found (Pl. XXIX, fig. 1, x), but further

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[1] Lacaze-Duthiers, 'Ann. Sci. Nat.,' ser. 4, vol. vi (Dentalium), and l. c., vol. xi (Pleurobranchus).

investigation has proved that this orifice leads into a sac which a complete series of sections have shown to be entirely closed. Fig. 4 shows the sac as seen when the pericardium is opened and one wall of the branchial vein removed. It is impossible to remove the whole of the branchial vein since one wall is closely adherent to the pericardial wall, with which is also fused one wall of the sac : the whole forms a very thin membrane (fig. 3, w, and fig. 5, w). The sac is lined inside by an epithelium. This does not form an even surface but is very irregular, dipping down into branched crypts ; the section chosen for fig. 5 passing through the orifice of the sac does not show these crypts.

The epithelium is of very different thicknesses in different regions, every here and there occur patches of a much thickened epithelium, and at other places the cells become very small indeed. A large number of the cells, more especially in the thickened patches, present glandular contents which stain deeply. I am inclined to think that the greater number of the cells in the thickened patches, perhaps all of them, are glandular, but those only show which happen to present contents. The whole surface appears to be ciliated, even the gland-cells. This may be, however, due to imperfect preservation, that is to say there may be smaller cells between the glandular cells which are richly ciliated and these may cause the whole membrane to appear ciliated. The structure of a portion of a thickened patch is shown in fig. 6, where a is a cell with glandular contents, m is the refringent margin which the cells present, p the slight amount of pigment which exists among the bases of the cells.

It is easy to understand how any injection might have ruptured the thin membrane dividing the lumen of the sac from that of the branchial vein, and so how Lacaze-Duthiers was led to the belief that here was a special communication between the exterior and the blood-vascular system. There is, however, no doubt that such does not exist.

What is this sac? Does it represent any structure found in other members of the group of Ctenidiobranchiate Palliate

Opisthobranchs to which Pleurobranchus belongs? Is it nephridial in nature? If it were it would be the rudiment of the second nephridium which persists in so few Gastropods (e.g. Fissurella, Patella) since there is a nephridium as well developed in Pleurobranchus as in Aplysia. This is not likely; its structure is not nephridial and I am convinced that it has no opening into the pericardium. Professor Lankester has suggested to me, and the view seems an extremely probable one, that we have here the homologue of that grape-shaped gland in Aplysia which has been long known as the " poison-gland." The position of the orifice of this gland is shown in fig. 2, *x*, and corresponds precisely in position with that of my sac.

Whether this suggestion as to the homology of the organ prove correct or not, it is I think quite certain that the special means of communication between the vascular system and the exterior, which has always been stated to exist in Pleurobranchus, has no existence.

EXPLANATION OF PLATE XXIX,

Illustrating Dr. A. G. Bourne's Paper "On the Supposed
Communication of the Vascular System with the Exterior
in Pleurobranchus."

Fig. 1.—Pleurobranchus testudinarius. Dorsal view of a specimen
from which the mantle-flap and a portion of the dorsal integument have been
removed by cutting along the surface *m*. *per*. Pericardium. *vent*. Ventricle.
aur. Auricle. *an*. Anus. *br*. ctenidium (gill). *g*. External genital organs.
x. Orifice of the glandular sac. *re*. The orifice of the renal sac lies in this
direction underneath the gill.

Fig. 2.—Aplysia limacina. Similar view. Other letters as in Fig. 1.
x. Orifice of the grape-shaped gland. *g*. Genital groove. *g. p*. Genital pore.

Fig. 3.—Diagram of the relation of the glandular sac to the pericardium
and auricle. *gl*. The glandular sac. *x*. Its orifice. *br*. The ctenidium.
br. v. The branchial vein. *au*. The auricle. *v*. The ventricle. *ao*. The aorta.
per. Pericardium. *w*. The wall separating the lumen of the glandular sac
from that of the auricle and branchial vein, composed in reality of three layers,
the wall of the sac, the pericardial wall, and the auricular wall, which together
form a single exceedingly thin membrane.

Fig. 4.—The glandular sac, as seen from the lumen of the auricle. *x*. Its
orifice.

Fig. 5.—Longitudinal section of the sac passing through its orifice *x*.
ep. Epidermis. *c*. The neck of the sac. *gl. ep*. The glandular epithelium
lining its walls. *a*. Gland-cells, showing deeply-stained contents. *c. t*. Con-
nective tissue. *w*. The exceedingly thin wall separating the lumen of the
sac from that part of the auricle.

Fig. 6.—A portion of the glandular epithelial wall of the sac more highly
magnified. *a*. Gland-cells with contents. *b*. Other cells. *ci*. Cilia. *m*.
Highly refracting margin. *p*. Pigment. *c. t*. Connective tissue.

Fig. 1.

Fig. 5.

Fig. 4.

Fig. 2.

Fig. 3.

Fig. 6.
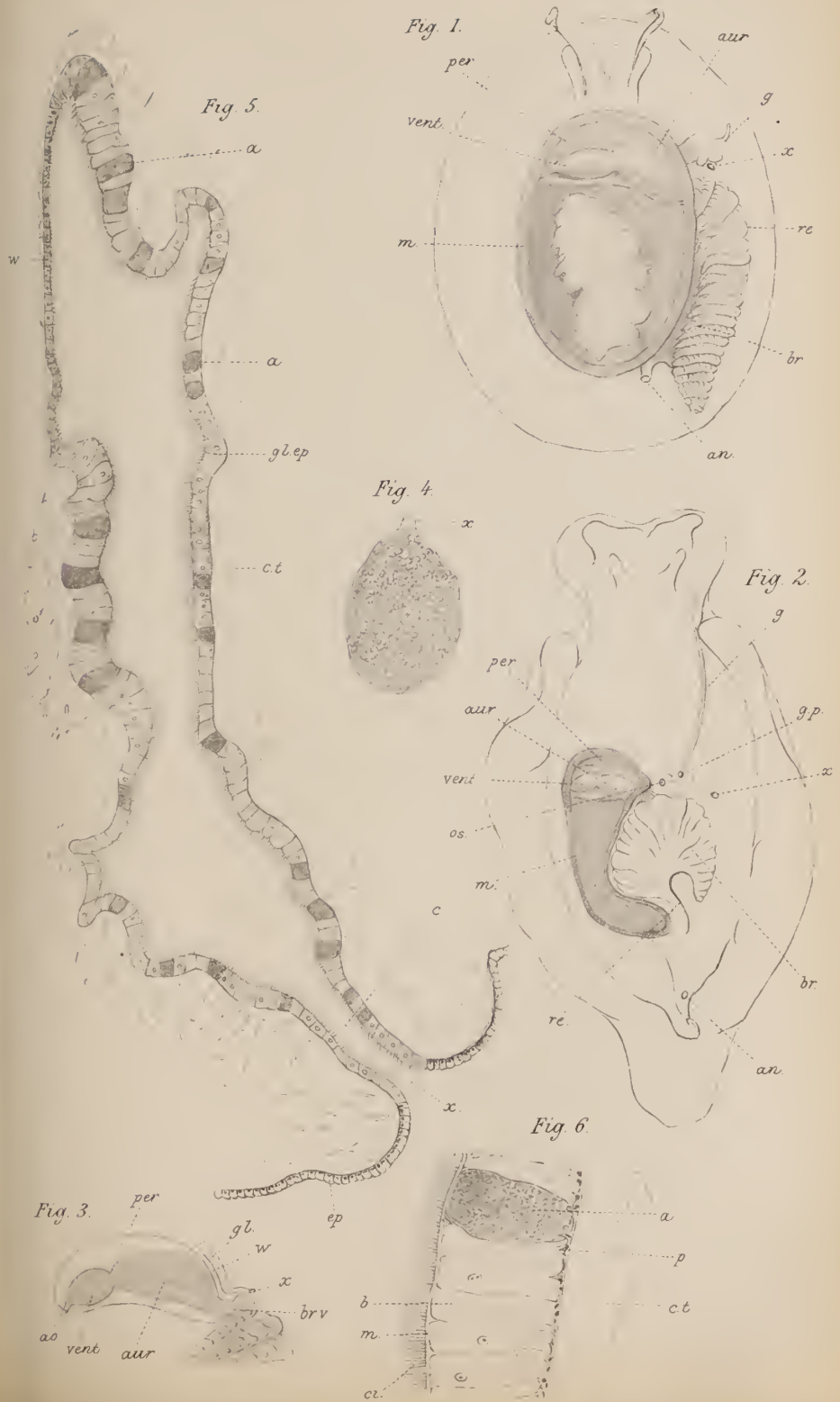
Observations on the Nervous System of Apus.

By

Paul Pelseneer, B.Sc.

With Plate XXX.

THE nervous system of Phyllopods recalls, by its appearance, that of some Chætopods. The two lateral cords are rather distant from one another, and the commissures which join the corresponding ganglia are rather long in the anterior part. Zoologists agree to recognise that, among the living Crustacea, the Phyllopods show the most primitive condition of the nervous system, and that this has remained in a rather archaic condition. Its study will be, therefore, for the explanation of some morphological facts, more useful than that of the nervous system of superior Crustacea, which has undergone many alterations.

The anterior part of the nervous system of Apus shows, when dissected, the following external appearance (fig. 1) : From the upper part of the brain C come the optic nerves N O, and from the lower part the two abdominal cords C A. From the latter, and rather far behind the brain, come, one after the other, the nerves of the two pairs of antennæ (A I and A II).[1] A little further elongated swelling

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[1] The two pairs of antennæ always exist in Apus ; this fact being already stated by Prof. E. Ray Lankester (this Journal, 1881, p. 346). Claus mentions the absence of the second pair of antennæ as characteristic of the Apusidæ ('Grundzüge der Zoologie '). As for me, I always saw the second pair of antennæ, even in large specimens of Apus, measuring 3·5 cent. from the head to the extremity of the abdomen. The first pair has, in these specimens, a

R A is found in each cord; then come, in succession, the mandibular and maxillary ganglia, the maxillipedal nerves, which are observed coming out of the cord like the antennary nerves, and the ganglion of the first thoracic foot.

Professor Ray Lankester has already pointed out the peculiar disposition of this nervous system, and when I came, during the winter of 1884 to 1885, to work under his direction, he urged me to study the subject. I take the opportunity to thank him here for the courteousness with which he received me in his laboratory, and for the good advice which he gave me during the time I spent in London.

In the course of my researches I have tried to elucidate the different points to which Professor Ray Lankester had drawn the attention of zoologists; they are the following:

I. The antennary nerves issue from the abdominal cord between the brain and the elongated swelling. Does this swelling arise from the fusion of the first and second ganglia? Or have the ganglia of these two appendages entirely disappeared, like the maxillipedes' ganglion, instead of fusing with others?

II. A pair of postœsophageal ganglia (the elongated swellings) exist anteriorly to the mandiblular ganglia, which are generally considered as the first ganglia of the abdominal cord.

III. Among superior Crustacea the brain gives birth to the nerves of the two pairs of antennæ. If we consider the nervous system of Crustacea as finally formed by two more or less distant lateral cords upon which is found a ganglion for every appendage, and a special cerebral ganglion-pair in which the two cords unite in front of the mouth, then the brain of supe-

length of 2·3 mill., the second of 0·9 mill. It is generally difficult to notice the second pair when one does not know where it is situated. Its position is not exactly shown by Zaddach ('de Apodis cancriformis anatomia et historia evolutionis,' pl. i, fig. 5, B); its insertion, in comparison with the first, is external, not internal.

rior Crustacea is to be regarded as formed by the fusion of the ganglia situated before the mandibular ganglion, a fusion which is connected with the welding of the cephalic segments. In Apus the optic nerves only are seen coming out of the brain. There are then good reasons to think that this brain is a simply primitive brain.[1]

IV. Since one sees the nerves of the two pairs of antennæ of Apus coming, not out of the brain, but rather far behind it, out of the abdominal cord, the two pairs of antennæ are metastomial appendages, which, in superior Crustacea, became prostomial in consequence of further displacement.

I shall now examine successively these different questions :

I. The first antennary nerve does not immediately turn forward when it comes out of the cord as it is shown in Zaddach's figure (loc. cit., pl. iii, fig. 5, *b*). On the contrary, it goes from forward backwards, showing a curve of a quarter of a circle, and going finally forward. This disposition is easily understood when one examines with a small magnification that part of the nervous system of Apus. One may then observe (fig. 1) that the fibres composing the first antennary nerve (A I), after having entered the abdominal cord (C A), proceed from behind forwards, and then towards the brain (C). They may be followed very far and teazed away from the other fibres composing the cord. At the point where the nerve joins the cord, one remarks upon the internal side of the latter, between the envelope and the nervous fibres, a large nerve ganglion-cell (C N) which has no relation with the first antennary nerve (A I).

The ganglionic cells from which this nerve comes off are not to be found in the elongated ganglion (R A, fig. 1) but in the brain. Therefore, if the latter is an archicerebrum, the

[1] Professor Ray Lankester calls these brains when formed by the single pair of primitive cephalic ganglia, archicerebrum, in opposition to the syncerebrum, or complex brain formed by the union of several pairs of ganglia ("Observations and Reflections on the Appendages and on the Nervous System of Apus cancriformis," this Journal, 1881).

first antennary nerve proceeds from the primitive cephalic gan-
glion; if, on the contrary, the brain comprises the first anten-
nary ganglion it is a syncerebrum.   It will be stated further
on, under No. III, that the latter hypothesis is the true one.

The second antennary nerve, when coming out of the cord,
goes immediately forward.   In examining that part of the
nervous system under a low power of the microscope, one
observes the following disposition (fig. 2): the fibres of the
second antennary nerve go into the abdominal cord (c A) from
in front backwards, towards the elongated swelling (R A).   The
latter is situated upon the internal side of the cord and pro-
trudes rather strongly.   In the interior of the cord, towards
its external side, one observes another smaller group of nerve-
cells (G A).   It is a small ganglion, composed of a few cells,
and there the fibres of the first antennary nerve end.

From the preceding statements it results :

1st. That the elongated ganglionic swelling does not repre-
sent the fused ganglia of the first and of the second antenna.
2nd. That the two pairs of antennary ganglia have not disap-
peared; they still remain in a very distinct manner.   The
second antennary nerve comes indeed from a small ganglion
situated in the cord in an external position to the elongated
swelling; and we shall see further on that the fibres of the
first antennary nerve come from a special and distinct ganglion,
situated on the posterior part of the brain.

As for the maxillipede nerve, its fibres go to a small ganglion
which is found in the cord a little in front of the first thoracic
foot ganglion.   Therefore, as in the case of the two antennæ,
the ganglion has not disappeared.

II. The ganglionic elongated swelling (R A) is joined to its
homologue by a double commissure, like all the ganglia of
the ventral cord.   According to Zaddach (loc. cit., pl. iii,
fig. 5, E), it seems that the anterior commissure comes out of
the stomatogastric nerve.   But, when dissected (fig. 2), both
the stomatogastic nerve (N s) and the commissure (c I) are seen
coming out of the elongated ganglion.

The homologue of the latter exists also among Decapods.

It is the small ganglion situated between the brain and the mandibular ganglion, a little before the first postœsophageal commissure.   To that ganglion come, on one side, the fibres of this commissure; on the other, the fibres of the stomatogastric nerve. In another Phyllopod, a neighbour of Apus, Limnetis, I think this ganglion may be seen in the swelling in front of the mandibular ganglion, a little behind the ganglion of the second antenna.[1]

This ganglion has been considered as the first postœsophageal ganglion of the abdominal cord.[2]

I think that it cannot be homologized with the ganglia of the cord.   In consequence of the great development of the stomatogastric nerve, it is easily understood that a ganglion appears on the point where it joins the ventral cord.   We have not here a segmental ganglion; it is only an adventitious one. Its lateral position in Apus is a proof of this statement.   It belongs more to the enteric system than to the somatic.

Nevertheless there are, in the abdominal cord, ganglia situated before the mandibles.   They are : the ganglia of the second pair of antennæ, the existence of which we have just ascertained, and the ganglia that we shall see in the brain, and from which come the nerves of the first antennæ.

III.—It is necessary to study the histological structure of the brain of Apus, and the disposition of the ganglionic cells, to show if it is an archicerebrum or a syncerebrum.

The brain of Apus is small and thin, and can be studied by transparency.   But I tried the method of serial sections after embedding in paraffine, to obtain more exact and precise results.   I used as colouring Kleinenberg's hæmatoxylin.   I have made transverse (bilateral vertical) and longitudinal (an-tero-posterior horizontal) sections.   By means of Caldwell's microtome I obtained complete series of sections.   But, the brain of Apus being very small, I found some difficulty in obtaining vertical transversal sections, and I have used prin-

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[1] Grube, "Bemerkungen über die Phyllopoden," 'Archiv für Naturg.,' 1853.

[2] Gegenbaur, 'Manuel d'Anatomie comparée,' p. 352.

cipally horizontal longitudinal ones. I have thus been obliged
to use a great many specimens.

The brain of A p u s is flattened and its shape quadrangular
(fig. 5). It is situated obliquely in relation to the longitudinal
axis of the body (fig. 3, c), and if one takes account of the dorsal
flexure of the nervous cord in front of the œsophagus,[1] the
brain's superior surface is in fact the ventral (infra-neural), and
the posterior margin out of which come the optic nerves is
the anterior margin.[2]

The ganglionic cells are accumulated on the ventral surface
of the brain; among these one can observe two distinct shapes:
first, small cells round or oval, forming a single layer on the
ventral surface (figs. 6 and 7, c s.) and a thicker mass on the
posterior edge of the brain (fig. 5, a p); secondly, large pyriform
cells which are found in certain parts of the brain.

I have drawn, cell by cell, the whole series of transverse
sections, so that I could clearly determine by superposition the
disposition of the ganglionic cells.

On the anterior part of the brain the large cells form a very
thick mass (fig. 4, a) at the beginning of the optic nerves.
That mass extends backward without transition, and assumes a
characteristic shape. In a transverse section taken a little
in front of the middle of the brain (fig. 6) the large cells, the
prolongations of which go toward the interior of the brain,
are divided into two symmetrical groups, distinct though joined
(g 1); they are the primitive cephalic ganglia.

If one goes on observing the successive transverse sections
from before backwards, the primitive cephalic ganglia will
be seen ending, without any continuation, a little after the
middle of the brain. Towards the edge of the latter, and princi-
pally towards the ventral surface, a second pair of groups of
large pyriform cells will appear (fig. 7, g 2). This group pro-

[1] Such a flexure is found in many Crustacea.

[2] I shall hereafter speak of the brain of A p u s morphologically, not topo-
graphically, i.e. the surface which is superior when the brain is in s i t u will
be called ventral surface; the posterior margin, by dissection, will be the
anterior margin, and v i c e v e r s â.

ceeds as far as the posterior end of the brain (figs. 4 and 5, G 2).

We have there a true second pair of ganglia held in the brain. The cells which form it are not small cells of the superficial layer; they are large pyriform cells attaining some thickness. These two ganglionic masses do not belong to the primitive cephalic ganglia, like the mass at the beginning of the optic nerves; they are, on the contrary, far from them, and entirely separated (see fig. 6, G 1, and G 2). They are then wholly independent of the primitive cephalic ganglia.

What is that second pair of ganglia?

If one examines a longitudinal section near the ventral surface of the brain (fig. 8) one can see that the prolongations of the cells composing these ganglia proceed backward, and that the nervous fibres which come from them go into the cord on the side whence come the fibres of the first antennary nerve. We have, therefore, here the ganglion of this appendage.

Therefore the brain of Apus is a complex brain, a syncerebrum formed by the juxtaposition of two pairs of ganglia, the formative elements of which remain separate. These two pairs of ganglia are the primitive cephalic ganglia and the ganglia of the first antennary pair, or first pair of the abdominal cord.

This proves obviously that the second pair of ganglionic masses in the brain is a pair of the abdominal cord, which has moved into juxtaposition with the primitive cephalic ganglia. These two groups (G 2, fig. 5) are not joined, as is the case for the last (G 1); they are very far from one another, and situated on the external edges of the brain, showing the same disposition as the ganglia of the abdominal cord. A horizontal section passing through one of the thoracic ganglia (fig. 9) shows the ganglionic cells situated on the lateral edges, just as is the case with the second pair of ganglionic masses of the brain.

As for the group of small cells situated on the posterior edge of the brain (fig. 7, A P), I do not give to it a special morphological signification. It is only a part of the superficial invest-

ment which has here a greater thickness. That mass seems to be common to the brains of all Crustacea, both inferior (B r a n-c h i p u s) and superior (A s t a c u s), and is also to be found on the anterior and posterior edges of the abdominal ganglia (fig. 9, A A and A P).

IV. The second antennary ganglion is situated next the first postœsophageal commissure (fig. 2). Its position determines in consequence this appendage as equally postœsophageal. There cannot remain any doubt about the second antenna; it is a metastomial appendage.

Is it the same with the first antenna?

Yes it is. The point where its nerve comes out of the abdominal cord evidently shows that the ganglion correspond-ing to that appendage must originally have been found far behind the prostomial cephalic ganglia (properly called cerebral ganglia). If it were not so the nerve, instead of coming out of the cord, would come out of the posterior part of the brain, as among other Phyllopods, B r a n c h i p u s, e. g. Besides, we have seen that the ganglia from which come the anterior anten-nary nerves have the structure of the abdominal ganglia, and therefore that they are ganglia of the abdominal cord. They were then originally postœsophageal, as well as the ganglia of the posterior antennæ. The corresponding appendages are thus also metastomial appendages.

We observe, then, that in A p u s, in the anterior (prægenital) part, every pair of appendages, the two pairs of antennæ, and the maxillipedes included, is provided with a pair of ganglia. Morover, the two pairs of antennæ are metastomial, as well as the following appendages.

We observe, too, that every ganglion has a kind of attraction, not only upon its homologue of the other cord, but even upon the preceding and following ganglia situated on the same cord. We see thus that the ganglia of the anterior antennæ run all along the cord to come into juxtaposition with the primitive cephalic ganglia; we observe also that the ganglia of the pos-terior antennæ pass to a place near the elongated ganglia of the stomato-gastric nerve, and that the maxillipede's ganglia

take up a position near those of the first thoracic feet. The two last pair of emigrated ganglia, reduced to a small size in consequence of the rudimentary condition of the corresponding appendages, have lost every sign of a commissure (fig. 2).[1] The ganglia of the anterior antennæ show, in the brain, nervous fibres which unite them with one another.

The facts we have observed in Apus, in regard to the antennary nerves, do not constitute an isolated case in contradiction with what is remarked among other animals of the same class. If one compares the nervous system of Apus with that of other Crustacea, a great concordance is found on the contrary.

Let us take a type in every family of the Branchiopodous Phyllopods. In Limnetis[2] the first antennary nerve comes equally out of the cord, but immediately behind the brain. Its ganglion is evidently held in the brain, just as in Apus. The posterior antennæ have a distinct pair of ganglia joined by a postœsophageal commissure.

In Branchipus[3] the nerve of the anterior antenna arises at the posterior part of the brain out of a group of cells distinct from the primitive cephalic ganglia; the nerves of the posterior antennæ, just as in Limnetis, come out a pair of cellular groups joined by a postœsophageal commissure.

Among Cladocerous Phyllopods Daphnia[4] shows a condition very like that of Branchipus.

In the Amphipod Phronima,[5] the nerves of the second pair of antennæ are also observed coming out of the abdominal cord, a little behind the brain.

[1] The two first postœsophageal commissures belong to the elongated ganglia of the stomatogastric nerve.

[2] Grube, " Bemerkungen über die Phyllopoden," ' Archiv für Naturg.,' 1853.

[3] Claus, " Zur Kenntniss des Baues von Branchipus, &c.," ' Abhandl. der K. Gesellsch. der Wissensch.,' Göttingen, 1873.

[4] Claus, " Zur Kenntniss der organisation der Daphniden," ' Zeitschr. für wiss. Zool.,' t. xxvii.

[5] Claus, " Der Organismus der Phronimiden," ' Arbeiten aus dem Zool. Inst. Wien,' t. ii.

We observe, then, that from the Crustacea with the most primitive nervous system to the most superior forms there are a great many transitions (Apus, Branchipus, Daphnia, Phronima, Astacus) between the condition in which the nerves of the two pairs of antennæ come out of the cord, and the condition in which these nerves come out of the brain.

Since then a histological examination shows in Apus, which among the actual Crustacea has the most primitive nervous system, that the brain is a syncerebrum, and since among superior Crustacea the anatomy of the nervous system shows that the brain is at least as complex as in Apus, it seems very probable that among living Crustacea there are not any with an archicerebrum.

The classification of the brains of Crustacea established by Packard[1] has no grounds whatever.

[1] 'American Naturalist,' 1882.

EXPLANATION OF PLATE XXX,

Illustrating Mr. Paul Pelseneer's Paper on "Observations on the Nervous System of Apus."

Fig. 1.—Left half of the anterior part of the nervous system of Apus. c. Brain. N o. Optic nerve. c a. Abdominal cord. a i. Nerve of the first antenna. a ii. Nerve of the second antenna. R a. Elongated swelling. c N. Nervous cell.

Fig. 2.—Beginning of the second antennary nerve. c a. Abdominal cord, a ii. Nerve of the second antenna. G a. Ganglion of the second antennary nerve. N s. Stomato-gastric nerve; R a. Elongated swelling (ganglion of the stomato-gastric nerve). c 1 and c 2. Anterior and posterior commissures of the elongated swelling.

Fig. 3.—Anterior part of the nervous system of Apus, side view. c. Brain. o. Right eye. o i. Median (larval) eye. c a. Abdominal cord. b. Mouth. T G. Digestive canal. a i. Beginning of the first antennary nerve. a ii. Beginning of the second antennary nerve.

Fig. 4.—Vertical projection of the brain of Apus.[1] o. Right optic nerve. o i. Median (larval) eye. a. Anterior cellular mass. G 1. First right ganglionic group (primitive cephalic ganglion). G 2. Second right ganglionic group (ganglion of the first antennary nerve). c a. Abdominal cord.

Fig. 5.—Horizontal projection of the brain of Apus. a P. Posterior mass of small cells. o i. Nerves of the larval eye. 6—6. Line showing the direction of the section drawn in Fig. 6. 7—7. Direction of the section drawn in Fig. 7. Other letters as for Fig. 4.

Fig. 6. Transverse section of the brain of Apus, passing through the line 6—6, Fig. 5. G 1. First ganglionic group (primitive cephalic ganglion). c s. Superficial lining of small cells.

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[1] In this projection, and in the following, the thin layer of small cells forming the superficial coating of the brain has not been drawn (see c s, Figs. 6 and 7).

Fɪɢ. 7.—Transversal section of the brain of Apus, passing through the line F—F, Fig. 5.   G 2.   Second ganglionic group of the brain (ganglion of the first antenna).   F N. Nervous fibres, joining the two ganglia.   C S. As in Fig. 6.

Fɪɢ. 8. Left half of a longitudinal section of the brain of Apus.   F N A I. Nervous fibres of the first antennary nerve.   Other letters as in Fig. 4.

Fɪɢ. 9.—Horizontal section of a ganglion of the abdominal cord.   G G. The groups of ganglionic cells.   C 1 and C 2. Anterior and posterior commissures.   N A S. Upper anterior nerve.   N A I. Lower anterior nerve.   N P. Posterior nerve.   A A. Anterior mass of small cells.   A P. Posterior mass of small cells.
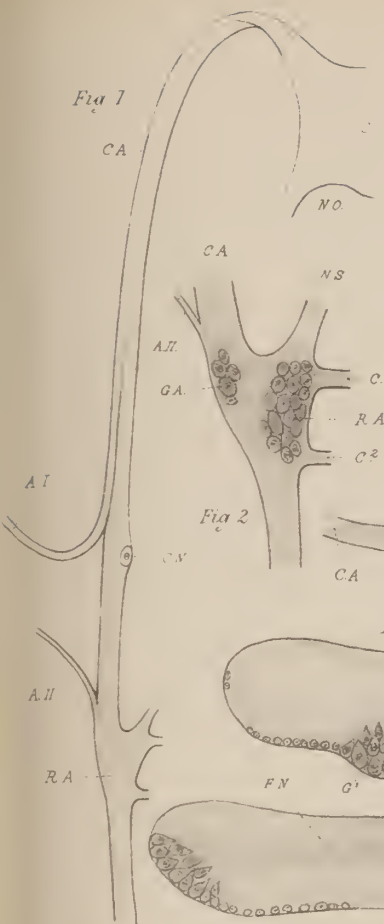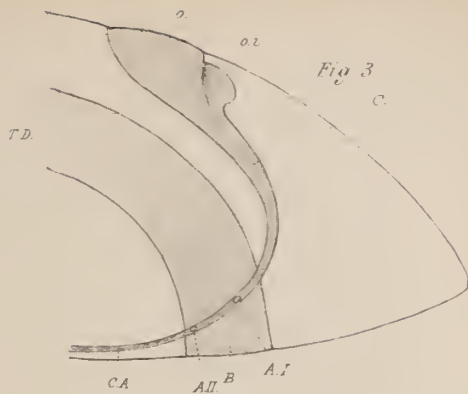
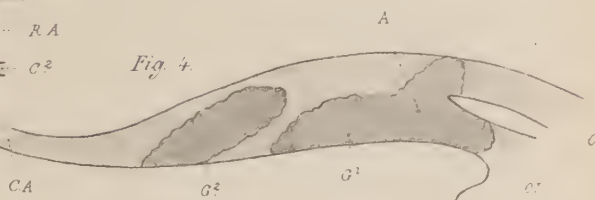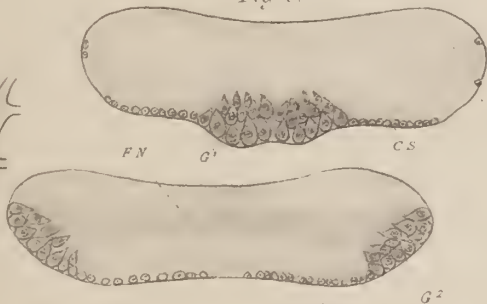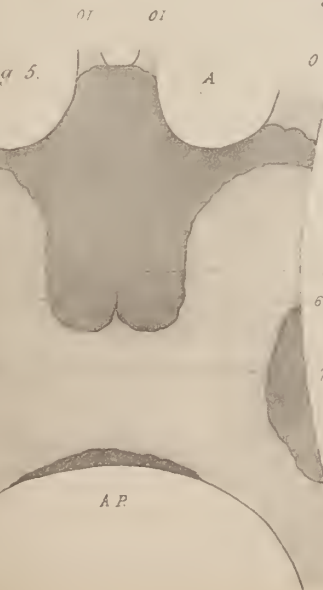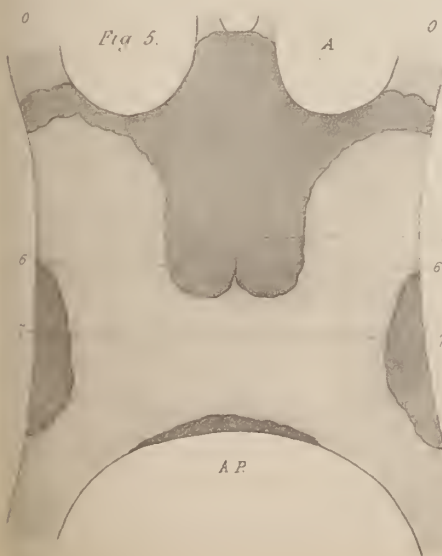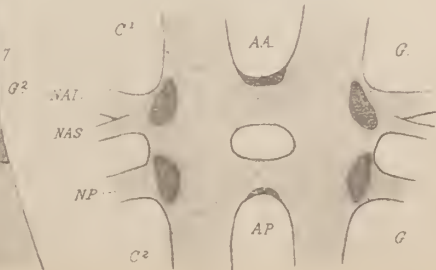# Note on the Chemical Composition of the Zoocytium of Ophrydium versatile.

By

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(From the Physiological Laboratory, University College, London.)

OPHRYDIUM VERSATILE is a ciliated Protozoon which grows in colonies or social clusters, exuding a common coalescent mucilaginous investing matrix or Zoocytium.[1]

Professor Lankester, to whom a large supply was kindly brought by Mr. Groom from the canal at Hereford, placed a quantity of this material in my hands with the object of determining the chemical nature of the jelly.

The lumps of jelly were on the average about an inch in diameter; the material was firm, colourless, and perfectly transparent. On its surface were patches of green due to the chlorophyll which is present in the animal itself.

The diagnosis of the material of which it was composed seemed to me to rest between mucin and cellulose; and it was found that the latter supposition was the correct one.

The percentage amount of solid matter in the jelly was found to be ·28 of which ·07 consisted of ash, and the remainder organic matter.

By digesting with warm water, a small amount of proteid material was extracted, doubtless contained in the protoplasm of which the animal itself is composed. This was removed by

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[1] Kent, 'Manual of the Infusoria,' vol. ii, p. 733.

digesting some hours with weak hydrochloric acid. The ash was found to contain the bases soda and lime, combined with chlorides, phosphates, and the merest trace of sulphates.

The basis of the jelly, however, was not nitrogenous; this fact, together with its absolute insolubility in lime water, baryta water, and other weak alkalies, showed that it could not be mucin.

The material was purified by successively washing it in cold water, hot water, dilute hydrochloric acid, dilute caustic potash, alcohol, and ether; it was insoluble in all these reagents, and the residue much shrunken by the action of the last-named reagents retained the shape of the original lumps.

It was then found to contain no nitrogen, and that it was cellulose was shown by the following properties that it possessed :

(1) It was insoluble in weak acids and alkalies.

(2) It was soluble in concentrated hydrochloric and sulphuric acids in the cold.

(3) The solution in sulphuric acid was diluted with distilled water and boiled for some hours; after this time it was converted into a sugar like dextrose which reduced cupric salts, and was capable of the alcoholic fermentation.

(4) With iodine and sulphuric acid it gave a yellowish-brown colouration.

(5) It was soluble to a slight extent in an ammoniacal solu- of cupric sulphate.

This substance then resembles vegetable cellulose in its general properties, and differs from it in being less easily converted into sugar. In this latter property it resembles tunicin, the substance of which the test of the Tunicata is composed; tunicin, however, is still more difficult to convert into dextrose, and according to Berthelot[1] requires some weeks boiling with dilute sulphuric acid to effect the change. Moreover, Berthelot says that tunicin gives a pale blue colour with iodine and sulphuric acid, resembling that given by cholesterin with

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[1] Berthelot, 'Ann. de Chemie et de Phys.,' série 3, tome lvi, p. 153.

the same reagents; the substance I examined gave a brown tint, so resembling some varieties of vegetable cellulose.

The proof of the existence of cellulose in the Ophrydium is interesting as showing that cellulose is not limited among animals to the Ascidian family; its coexistence with chlorophyll, another vegetable product, in the same animal is also noteworthy.

The Development of Peripatus Capensis.

By

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PART I.

With Plates XXXI and XXXII.

INTRODUCTION.

THE development of Peripatus capensis was first studied by Moseley,[1] who stopped for a short time at the Cape in November and December some years ago. His observations related only to stages which were comparatively late in development. Balfour, in 1882, found some younger embryos in specimens collected by Mr. Lloyd Morgan in July and August, and sent to Professor Huxley, who gave them to Balfour. He had only time to make a very few observations, of which he left a short record in the form of four rough drawings and a short note, and a letter to Professor Kleinenberg, before starting on his last expedition to Switzerland. His observations were so interesting that they were made the subject of a short communication to the Royal Society in the autumn of 1882, and they were slightly extended by the editors of his last work on the Anatomy of Peripatus capensis,' and published with that monograph in the 'Quarterly Journal of Microscopical Science' in the spring of 1883.

The subject seemed so important that the Government Grant Committee of the Royal Society granted, in the spring of 1883, the sum of £100 to enable me to go to the Cape for the

[1] 'Phil. Trans.,' vol. 164.

purpose of obtaining well-preserved embryos, and of studying the development on fresh specimens.

Accordingly, I went to the Cape in the summer of 1883, arriving early in July, and remaining till the middle of August. I obtained a large number of specimens, and brought back with me over 300 alive. Some of the latter lived at Cambridge till the following July. The results of my observations at the Cape and after my return to England have been to show that the embryos remain thirteen months in the uterus; that the fertilised ova pass into the uterus in April, and the young are born, fully developed, in the May of the year following. That is to say, the young ova pass into the uterus one month before last year's young are born. I was not prepared for this, and I did not, in 1884, examine my specimens for the early stages until May, when the young were being born. The result was that I missed last year the early stages of development, and had it not been for the kindness of Mr. Walter Heape, who went to South Africa last summer, and who collected and brought back some more live specimens, I should have been obliged to leave the early stages undescribed. Thanks to him, however, and to my experience gained last year, I have this April been able to find several of the younger stages, and to complete my observations.

Two species of Peripatus are commonly found at the Cape. One, the most common, is the well-known Capensis; the other is a new species, differing from Capensis in having eighteen pairs of fully-developed legs, in being of a smaller size, and in other points. This species I propose to call Peripatus Balfouri. It will be fully described in the forthcoming monograph by Moseley and myself on the 'Species of Peripatus.'

Besides the work of Balfour and Moseley on the development of Peripatus capensis, some observations on the development of a West Indian species have been published by Dr. J. Kennel, of Würzburg (Semper's 'Arbeiten,' Heft ii, Bd. 7). I do not propose to enter here into any detailed examination or criticism of Dr. Kennel's account of his observa-

tions, which relate almost entirely to the early stages.   Dr. Kennel has described his observations at very great length, but I do not think that his account of them can be regarded as entirely satisfactory.   It is quite clear that Dr. Kennel has been hampered by want of material in the early stages, and by the great difficulty of the subject, and I therefore defer a detailed examination of his work to a later paper in the series of which this forms the first, by which time I hope that he will have been able to throw some more light upon certain points in the early development of the West Indian species, which are left somewhat obscure in the first account in Semper's 'Arbeiten.'   On one point, however, there can be no doubt, viz. that the early stages in the development of the West Indian species are very different from those of the Cape species.   Whether they are as different as Dr. Kennel makes out I am inclined to question, but that, as I have said, is a matter for further elucidation.

Some of the more important results of my observations on the development of Peripatus capensis, e. g. the derivation of the mouth and anus from the blastopore, the fate of the grooves in the cerebral ganglia, have already, some time ago, been published in my paper "On the Origin of Metameric Segmentation" (this Journal, 1884).

My observations are nearly completed, and I have already (May of this year) communicated a preliminary account of them to the Royal Society.   They will be published in full, I trust, in a series of papers in this Journal.

The present paper is the first of the series, and relates almost entirely to the segmentation and general development of the embryo.   I have deferred all discussion of the facts described to a more convenient opportunity in a later paper in the series.

All the drawings in this paper, with the exception of figs. 23—27, have been made by Mr. E. Wilson, of the Cambridge Scientific Instrument Company.   They are very careful and accurate representations of the specimens, and I cannot sufficiently express my thanks to Mr. Wilson for the great trouble he has taken with them.

The segmentation stages were all drawn in the laboratory as I removed them from the uterus, and it is entirely owing to Mr. Wilson's kindness that I have been able to obtain a permanent and accurate record of the various stages of the living segmenting ovum.

The Generative Organs.

At the outset I must give a short description of the general arrangement of the generative organs. Their minute structure I shall describe more fully when I come to their development.

Male Organs.—The description given by Moseley[1] and by Balfour[2] is correct so far as the general arrangement is concerned, but a slight rectification is necessary in the significance to be attached to the various parts. The structures called testes by these authors (Balfour, loc. cit., Pl. XX, fig. 43, *te*.) are apparently merely seminal vesicles, in which the spermatozoa develop and gain maturity. The true testes are the so-called prostates (Balfour, loc. cit., fig. 43, *pr*.), the lining cells of which fall into the cavity of the tube, pass into the swollen seminal vesicle, where they develop into spermatozoa. The spermatozoa, when ripe or nearly ripe, pass into the vasa deferentia, at the lower end of which they apparently become packed together in small masses, which become surrounded by a structureless coat, and are passed out as small, oval, white spermatophores.

The male generative organs of Peripatus capensis consist, therefore, of a pair of blind tubes, which are separate in front, but united behind for a short distance to form a common terminal portion (Balfour, loc. cit., fig. 43, *p*), and at the front end of which are formed the mother-cells of the spermatozoa. The latter fall into the cavity of the tube, and gradually develop as they pass backwards towards the external orifice, the

[1] 'Phil. Trans.,' vol. 164.
[2] This Journal, 1883.

main portion of the development taking place in a specially
dilated portion of the generative tube—a portion which may be
called a vesicula seminalis.

I have never seen the extrusion of the common terminal
portion of the system, and I doubt very much whether it ever
is extruded so as to act as a penis.

The Female Organs.—The ovary, though apparently a single
structure, is in reality paired, and consists of two tubes closely
applied together. The ova are derivatives of the epithelial
lining of these tubes. Each ovarian tube is continued into the
oviduct of its own side. The oviducts are thin-walled, narrow
tubes, each of which is continued behind into a more dilated and
thicker-walled tube—the uterus. The uterus is of consider-
able length and much bent, and unites with its fellow close to
the single external opening, which lies in the middle line of
the ventral surface of the hind end of the body, just in front
of the anus. For figure of female organs, *vide* Moseley, loc.
cit., pl. 74, fig. 1, and Balfour, Pl. XIX, fig. 33.

From the above description it is evident that the organs of
the female, like those of the male, consist of two tubes united
behind near the external opening but ending blindly in front,
where the generative products are produced.

The ovarian parts of the generative tubes are placed between
the fifteenth and sixteenth pairs of legs, and are united to the
floor of the pericardium by a delicate band of transparent
tissue. They, i. e. the ovaries, contain spermatozoa, some of
which project through the ovarian walls into the body-cavity.
This condition has been figured and described by Moseley (loc.
cit., pl. 74, fig. 1).

The ovaries always contain spermatozoa, but in smaller
numbers directly after the eggs have passed into the ovi-
duct than at any other time. This is a very marked feature
of an ovary, say, of the beginning of April, when compared
with an ovary from which the ova have just passed into the
oviducts, say, of the beginning of May, the former being of an
opaque-white colour to the naked eye, while the latter has a
much more transparent appearance.

This fact would seem to imply that fresh spermatozoa pass each year into the ovaries. This brings me to the question of the manner in which the male discharges his function. The vesiculæ seminales (testes of Moseley and Balfour) are almost empty of spermatozoa in the months of February, March, and April. At the end of April, however, they begin to swell again and contain spermatozoa, which increase in number as time goes on, until, in October, they are fully distended with spermatozoa in all stages of development. There seems to be no functional intromittent organ, but the male deposits little oval spermatophores quite casually on any part of the body of the female, and, for all that I know, of the male also; e. g. I have often seen them on the head. How these little packets of spermatozoa get into the vagina, and then up the uteri, which are always full of embryos, I cannot conceive. The spermatozoa exhibit a certain amount of vibratory movement, and no doubt, once within the vagina, they are set free from the spermatophor and make their way up the female generative tube, between the embryos and the uterine walls. Inasmuch as the deposition of spermatophores lasts from June until January, each female probably has a large number of spermatophores deposited on her, and some of these are probably near the generative opening, and are, somehow or another, transported through it into the vagina.

Fertilisation is apparently effected in the ovary. I have never seen spermatozoa in any part of the female apparatus except in the ovaries, and in small numbers in the upper end of the oviducts at the time when the ova are entering the latter.

The ripe, and probably fertilised, ova pass into the oviduct in April, while the uterus is still full of embryos almost ready for birth. Segmentation and the early stages of development take place during the passage of the ova down the oviduct. In May the young of the previous year are born. Into the uterus, thus emptied, the young ova pass, and establish themselves in the positions which they maintain until the following May, when they are born.

The passage of the ova down the oviducts and uteri is effected by the peristaltic contraction of the walls of these structures.   I have never been able to see cilia in the generative organs, or in any other part of the body of Peripatus capensis.[1]

The living ripe ovarian ovum is somewhat elliptical in shape and of dark colour by transmitted light.   The opacity is due to the presence of granules, which are uniformly distributed in the protoplasm, but absent altogether from the larger germinal vesicle.

As I have stated above, I propose to defer my account of the ovary and ovarian ovum until their development is considered.

The Fertilised Ovum.

The youngest ovum found in the oviduct is shown on Pl. XXXI, fig. 1.   It is of an elongated form—length ·4 mm. —and is surrounded by a transparent, structureless membrane, which is either a vitelline membrane or derived from the follicular cells surrounding the young ovarian ovum. This membrane persists until birth; it has a dense structure and allows fluid to pass through it by diffusion.   Water diffuses through it more rapidly than alcohol, and alcohol more rapidly than turpentine; so that when an embryo is removed suddenly from weak alcohol into strong, or from absolute alcohol into turpentine, the membrane shrinks and closely invests the embryo; in fact, in the latter case all the alcohol diffuses out before any turpentine enters, so that the membrane completely collapses and squashes the embryo flat. When, on the other hand, an embryo is removed from strong alcohol into weak, or into water, the water passes in more rapidly than the alcohol passes out, so that the membrane is distended, and the space between it and the embryo much enlarged.   In the normal embryos there always is a space between the membrane and the ovum, which contains fluid in

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[1] This remark applies to the nephridia, all parts of which I have carefully examined in the fresh state without ever seeing a trace of cilia.

which the embryo floats.   The membrane, therefore, has much the same function as the amnion of the Vertebrata.

The protoplasm of the ovum [1] is differentiated into two parts— the main mass being of a pale colour with relatively few dark granules, while at one point it is especially dark in colour. This small dark patch (fig. 1) is placed at the surface on one of the long sides of the ovum.   I may call it, from its position as determined by the later development, the dorsal or animal pole of the ovum.   When the ovum is viewed from the side (fig. 1), it is seen that the surface of the dark patch is pitted inwards, and that the space so formed contains two small clear bodies, which I take to be polar bodies.   When viewed from the face, the dark patch presents a central circular transparency more or less free from the dark granules which are found in such large numbers in other parts of it.   This central clear body I take to be the first segmentation nucleus.   The polar bodies are only seen during this stage, and I have no observation either on their origin or fate.

I have figured two other unsegmented ova (figs. 2 and 3) which differ in certain respects from the above.   In one of these (fig. 2) the dark patch is smaller than in fig. 1, and without the central transparent area ; in the other (fig. 3) there were several dark patches, each with its own clear spot.   I have not been able to make out the meaning of these differences, i.e. whether they are normal stages in the development of the ovum, and if so, where they come in the developmental series.

Segmentation.

Segmentation is complete.   The first furrow is in the transverse plane of the ovum, and divides it into two halves (fig. 4), the dark patch being divided as well as the main mass of the ovum.   The second furrow is at right angles to the first, and divides each of the first formed segments into two (figs. 5 and 6), so that the ovum now consists of four segments, each consisting of a lighter-coloured main mass and a small dark patch

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[1] The following description of the segmenting ova refers, unless otherwise stated, to fresh living ova seen in transmitted light.

which closely adjoins the dark patches of the three other cells at the animal pole, and which contains a central clear area (fig. 5).

The two first furrows, therefore, are at right angles to one another, and in the vertical plane. The next furrow is horizontal, and divides each of the four segments into two unequal parts; a small animal part consisting almost if not entirely of the dark animal part of the cell, and a larger, clearer mass; so that the ovum now consists of eight cells, four small cells lying close together at the animal pole, and consisting almost entirely of the darkly granular parts of the cells of the previous stage, and four large, clearer, but more or less granular cells. Each of the small dark cells contains a central transparent area, which I take, as before stated, to be the expression of the nucleus.

The four small dark cells give rise to the ectoderm, and the four larger cells to the endoderm.—The subsequent division of these two kinds of cells proceeds independently.

In the next stage I have figured (fig. 7), there are eight dark cells, each with its central clear area, and an undetermined number of large endoderm cells.

At the end of segmentation (fig. 8), the ovum consists of a number of large-branched endoderm cells scattered irregularly within the egg membrane, while the ectoderm cells consist of a mosaic of more or less hexagonal cells closely applied together and placed close to the membrane on one side at about the middle of the long axis of the egg.

The egg at this stage presents a very peculiar appearance, and I would not believe for some time that I was not dealing with an abnormal or injured ovum. But I found the stage so often, and so many stages intermediate between it and the earlier and later stages of development, that I cannot but believe in its normal existence. I found it also when every precaution was taken to avoid injuring the ovum; when I merely opened the animal and examined the ovum through the transparent walls of the oviduct without even touching any part of the female organs.

The endoderm cells at this stage—and I have no doubt this is the case in other stages, but in this case the fact can be clearly seen—are branched, and the branches of adjoining cells in some cases anastomose. One must suppose in fact that the endoderm cells of this stage are amœboid and capable of independent movement, in order to account for the changes which now take place.

In fig. 9 I have had drawn an embryo of this stage as an opaque object, with reflected light. The drawing shows clearly the mosaic of ectoderm cells, which in this light seem to be composed of a brilliant white substance with a central dark area.

The endoderm cells now begin to draw together towards the centre of the egg, and come to lie directly beneath the ectoderm mosaic, which rests upon them like a cap. I have had various stages of this process drawn in figs. 10—14.

This change can only be explained as being due to an active movement of the endoderm cells, which travel from all parts of the egg towards the centre, where they aggregate in masses which gradually unite with one another, forming at first a ring and then uniting further until they form one more or less spherical solid mass of cells on which the ectoderm mosaic rests like a cap. Fig. 15 shows a side view of an embryo at this stage, in which this process of aggregation of the endoderm cells is completed. Fig. 15 is drawn from a preserved embryo made transparent by turpentine.

The nucleus of the ectoderm cells, which has been conspicuous in all these stages by its transparency and freedom from granulations, presents quite a different appearance in embryos which have been treated with reagents. In the latter case instead of a central transparency, we find a central mass of dark granules, which are much more marked than the granules of the body of the cell. Further, it should be pointed out that, in the latter stages, the granulation of the ectoderm cells is a much less marked feature (*vide* figures), and that the boundary between the ectoderm cells becomes less distinct (*vide* especially fig. 13).

The ectoderm now grows round the endoderm cells and entirely surrounds them, excepting at one point.   At this point, which is opposite to that on which the ectoderm cap was placed, the endoderm cells may be seen for a short time projecting (figs. 17 and 18).

The embryo has thus acquired a spherical form, and consists of a solid gastrula, the small uncovered spot of endoderm constituting the blastopore.   A cavity next appears in the centre of the endoderm cells, so as to open to the exterior through the blastopore (figs. 19 and 21).

We have thus arrived at the stage of a typical gastrula formed of two layers of cells, which are continuous with one another at the blastopore and enclose a central cavity.   It may be at once stated that the blastopore, which is on the ventral surface of the embryo—on the surface opposite to that on which the ectoderm cap was placed—persists and gives rise to the mouth and anus[1] of the adult, and that the cavity of the gastrula becomes the mesenteron.

The General Development of the Embryo.

The segmentation, as we have seen, is complete but unequal; the large cells giving rise to the endoderm, and the small cells to the ectoderm.   The gastrula arises by a modified process of epibole.   The fully-developed gastrula is shown in figs. 19 and 21.   The embryo has already become slightly oval, and the blastopore now begins to elongate in the direction of the long axis.

Stage A (fig. 22).—An opacity appears at the hind end of th blastopore.   This opacity is the primitive streak.   It appears to be due to the active proliferation of some cells, which cannot

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[1] These were called in Balfour's memoir (this Journal, 1883), and perhaps more correctly, the embryonic mouth and anus,—more correctly because they come in the adult to lie internally, in consequence of the ingrowth of ectoderm at the two ends of the alimentary canal to form the stomodæum and proctodæum.   They constitute in the adult the openings between the mesenteron and the stomodæum and proctodæum respectively.   It must, however, be borne in mind that they never become closed.

be definitely assigned either to the ectoderm or the endoderm, at the hind end of the blastopore. This stage, which has already been described in Balfour's Memoir on Peripatus (this Journal, vol. xxiii), is found most commonly at about the middle of June in England.

The embryo now grows considerably in length (fig. 23), the blastopore presenting a corresponding elongation, and the mesoderm, which arises from the proliferation of the undifferentiated cells of the primitive streak, grows forward as two ventrolateral bands, one on each side of the blastopore.

The mesodermal bands next divide by transverse division from before backwards into somites, which contain a cavity, part of the future body cavity. The first somite to appear is the anterior, and then successively backwards.

Stage **B** (fig. 25).—The blastopore now divides into two parts (figs. 24 and 25) by the obliteration of its median portion —into an anterior part which becomes the mouth of the adult, and a posterior part which is at first placed at some little distance from the hind end of the embryo and gives rise to the anus of the adult.

The primitive streak still persists and extends from the hind end of the blastopore to the hind end of the embryo. It is now marked by a groove—the primitive groove (fig. 25).

The anterior pair of somites have shifted forward to quite the anterior end of the body ; they give rise to the mesoderm and body cavity of the præoral lobes.

Stage **C** (figs. 26 and 27).—The hind end of the body now becomes curved ventrally (figs. 26 and 27). The curve is produced by the growth of the hind end of the body. As this growth proceeds the curve becomes more marked, and assumes a spiral form, that is to say, the hind end of the body is spirally coiled, the coil being applied to the ventral face of the anterior part of the body (fig. 28).

Stages B and C are found in July and August at the Cape.

Stage **D** (figs. 28 and 29).—The spiral stage is characterised by

the appearance of the appendages and of the lip-fold which encloses the jaws in the adult, and of the eyes.

The appendages arise as hollow processes of the body wall, containing prolongations of the somites. The first to appear are the antennæ, into which the præoral somites are prolonged. The remainder appear from before backwards in regular order, viz. jaws, oral papillæ, legs, 1, 2, . . . 17, and the rudimentary anal papillæ, which are the appendages of the last, i. e. the twenty-first somite.

The full number of somites and their appendages is not, however, completed until a later stage, the posterior being the latest to appear.

The eyes appear in this stage as invaginations of the sides of the nervous thickenings (the future supraœsophageal ganglia) of the præoral lobes (fig. 29, e). The invaginations are at first shallow, but soon become deeper, and in the next stage converted into closed vesicles, the front wall of which (i. e. the wall next the skin) forms the epithelium outside the so-called lens of the adult eye, while the internal wall thickens, and remains continuous with the cerebral ganglion, and gives rise to the retina. The enclosed vesicle persists, and apparently becomes filled by the structureless lens of the adult eye, if the structure described as such be not a mere coagulum produced by reagents. The eye of Peripatus is therefore a cerebral eye.

The lips.—The end of the spiral stage is also characterised by the appearance of the buccal fold or fold which encloses the jaws and buccal cavity, and so constitutes the tumid lips of the adult. This is a fold of the side walls of the body immediately outside the jaws, and extending from the præoral lobe of its side to just behind the jaw. It is at first most marked in front, which fact led Moseley to describe it as a backward process of the præoral lobe.

The first indication of the lip is shown in fig. 29, just behind the eye; it is seen better, however, in the figure of the next stage (fig. 30, L).

This stage is also characterised by the fact that the anus has shifted to the hind end of the body (the primitive streak

having apparently disappeared). The præoral lobes have also become markedly bilobed as compared with the previous stage (fig. 26).

Stage E (figs. 30—34).—In the next stage (fig. 31), which is found early in October in England, the spiral straightens out, and the embryo becomes bent double, the ventral surface of the hind part of the body being applied to the ventral surface of the front portion, and the tail end of the embryo being curled round the front end of the head. The bend occurs at the level of the eighth somite.

An embryo straightened out and drawn from the side is shown in fig. 30.

The main features of this stage, in addition to the loss of the spiral form, are—(1) the increase in the number and size of the somites and appendages; (2) the closure of the eye-pits; (3) the growth of the lips; (4) the appearance of a groove in the thickened ectoderm on the ventral surface of the præoral lobes; (5) the presence of a well-marked dorsal projection at the level of the anterior bend of the body; (6) the beginning of the ectodermal invagination which will form the stomo-dæum; (7) the appearance of a pit at the apex of the oral papilla (fig. 33, *or. p.*); and (8) of a perforation on the hinder part of the ventral swellings at the base of the oral papillæ (fig. 33, *o. s.*).

With regard to these points, I may make the following observations:

1. The antennæ have become ringed, and the number of somites is almost completed. There are ultimately twenty-one somites; in this specimen twenty could be made out (fig. 32).

2. In the specimen figured (fig. 30) the eye-pits were not closed; they remained open in this specimen abnormally late.

3. The lip-fold has grown considerably, and extended on to the ventral surface behind the jaws (figs. 30, 33, *L.*).

4. These grooves are shown in fig. 35 (*c. g.*), which is taken from a young specimen of the next stage. They are at first wide and shallow, but, as we shall see, soon become deep and narrow, and eventually closed.

5. This projection had already appeared in the spiral stage (figs. 28, 29), but it first becomes conspicuous in this stage (fig. 30, *d.*).   It is placed at the level of the eighth somite, and consists simply of a thickening of the dorsal and lateral ectoderm.

6. This process is shown in its two first stages in figs. 33 and 34, *st.*   In fig. 33 the posterior margins of the buccal opening are beginning to grow in beneath the anterior margins ; the same feature being shown more clearly in fig. 34, *st.*

7. These pits are caused by an ectodermal invagination which will give rise to the slime glands.

8. These two perforations (fig. 33, *o. s.*) are actual perforations leading through the body wall into the body cavity of the third somite (somite of the oral papillæ) ; they become the external openings of the salivary glands.

Stage F (figs. 35, 36).—The next stage, which is also found in October in England, is very close to the previous one, and I have only thought it necessary to figure a ventral view of the head (figs. 35, 36).   Fig. 35 is from a specimen slightly younger than fig. 36, in fact from a specimen intermediate between this stage and the previous stage.   It has already been referred to as showing the grooves in the brain (*c. g.*), which first appear in stage E.   The main features of interest in this stage relate to the head and anterior somites.

(1) The lips have become very conspicuous and folded (fig. 36); they have extended on to the ventral surface, passing inwards between the jaws and oral papillæ, behind the openings of the salivary glands, which they have completely covered up, and finally have united with one another in the median ventral line, so as to form the posterior part of the adult lips.

Fig. 35 is especially interesting as showing an earlier phase in this growth.   In this figure the folds have not yet reached the middle line, and are still very inconspicuous behind the salivary openings (*o. s.*), which are still exposed.

(2) The cerebral grooves (fig. 36) have become much deeper, and their opening reduced to a narrow slit, ending behind in the mouth and slightly dilated in front.

(3) The ingrowth of ectoderm into the mouth-opening is completed in fig. 35. In fig. 36 the mouth-opening has become reduced to a narrow slit by the approximation of the ventral swellings at the base of the jaws (cf. figs. 35 and 36, *j. s.*).

(4) The præoral or cerebral lobes, which were distinctly bilobed and separate in the previous stage (fig. 33), have now again become quite continuous across the middle line (cf. figs. 33, 35, 36), a shallow groove only marking the original line of separation.

Stage G (figs. 37, 38).—The last stage which I have thought it necessary to figure and describe is found in England in December (figs. 37, 38). The differences between this and the previous stage consist mainly in the growth of parts already present.

The embryo is characterised by its great transparency. The full number of appendages is present, and the appendages have acquired more nearly their adult form. They are all ringed, and the rudiments of claws have appeared on the legs. The appendages (fig. 37) are antennæ, jaws (now completely hidden by the lips) oral papillæ, seventeen pairs of legs, and the small anal papillæ (*an. p.*).

The skin presents slight projections, shown as white opaque marks in the figure; these are the commencement of the papillæ, which cover the skin of the adult. The dorsal projection is still a conspicuous object (*d*), though not so conspicuous as in the earlier stages.

The integument presents a ringed appearance (fig. 38); the rings, however, have nothing to do with the segmentation of the body, being far more numerous than the segments.

The mesenteron is distinctly visible as a wide tube which behind passes into the narrow rectum (*R*). The rectum is probably lined by an ingrowth of ectoderm through the anus and may be looked upon as a proctodæum.

The salivary glands (*s. g.*) can be seen through the skin, and have grown some distance backwards. The same is to be said of the slime glands (*sl. g.*) which, however, are directed more

dorsally. The salivary glands are, as I have said in my preliminary paper, the nephridia of the third somite, i. e. the somite of the oral papillæ.

The remaining nephridia are also visible through the skin, those of the fourth and fifth legs being especially conspicuous by their greater size.

Fig. 38 represents this embryo in its natural position within the uterus, a position which is retained until birth.

From January onwards the changes are merely those of growth. When the young are born, i. e. in May, the antennæ are green, but the rest of the body is either quite white or of a reddish colour. This red colour differs, however, essentially from that of the adult, in the fact that it is soluble in spirit. The just born young vary considerably in size, the average size in the case of Peripatus capensis being from 10—15 mm. The just born young of Peripatus Balfouri are about half this size.

EXPLANATION OF PLATES XXXI and XXXII,

Illustrating Mr. Sedgwick's Paper on " The Development of Peripatus Capensis."

List of Reference Letters.

a. Anterior end.   *a.* Anus.   *an. p.* Anal papillæ.   *At.* Antennæ.   *c. g.* Groove in brain.   *d.* Dorsal ectodermal thickening.   *e.* Eye.   *ec.* Ectoderm. *en.* Endoderm.   *F.* 1 . . . . *&c.* Feet.   *j.* Jaw.   *j. s.* Swellings at base of jaws.   *L.* Lip.   *M.* Mouth.   *me.* Mesenteron.   *or. p.* Oral papillæ.   *o. s.* Opening of salivary gland.   *p. s.* Præoral somite.   *R.* Rectum.   *s.* 20. 20th somite.   *s. g.* Salivary glands.   *sl. g.* Slime glands.   *s. o.* 4 and 5. Nephridia of 4th and 5th legs.   *st.* Ectodermal ingrowths into embryonic mouth.

PLATE XXXI.

All the figures on this Plate, except 15, 17—22, are from fresh specimens.

Fɪɢ. 1.—Per. Balfouri. Side view of unsegmented ovum, showing polar bodies and dark patch. Greatest length ·4 to ·48 mm.

Fig. 2.—Per. Balfouri. Unsegmented ovum with dark patch, but without central clear spot.

Fig. 3.—Per. Balfouri. Unsegmented ovum with numerous dark patches, each with a clear centre.

Fig. 4.—Per. Balfouri. Ovum with two segments from side.

Fig. 5.—Per. Balfouri. Ovum with four segments from animal pole.

Fig. 6.—Per. Balfouri. Side view of ovum with four segments.

Fig. 7.—Per. Balfouri. Ovum with eight dark segments from animal pole. Greatest length ·4 to ·48 mm.

Fig. 8.—Per. capensis. Ovum fully segmented, with mosaic of ectoderm cells and scattered branched endoderm cells. Greatest length (of eggshell) ·56 to ·6 mm.

Fig. 9.—Per. Balfouri. View of ovum from animal pole, as opaque object.

Fig. 10.—Per. capensis. Aggregation of the endoderm cells beginning.

Figs. 11 and 12.—Per. Balfouri. Completion of same process.

Fig. 13.—Per. capensis. Illustrates the same point. Length of ectoderm patch ·32 to ·4 mm.

Fig. 14.—Per. capensis. Another phase of the same process.

Fig. 15.—Per. capensis. Side view of ovum from preserved specimen. Cap of ectoderm cells covering half the endodermal mass. Progress of epibole. Diameter ·240 mm.

Fig. 16.—Per. Balfouri. Stage in which the endoderm cells are covered by the (f.) latter ectoderm cells. Diameter ·32 mm.

Fig. 17.—Per. capensis. Side view of embryo. A few endoderm cells exposed.

Fig. 18.—Ventral view of same.

Fig. 19.—Per. capensis. Gastrula stage, ventral view. Blastopore distinctly circumscribed. Size ·204 mm. × ·240 mm.

Fig. 20.—Side view of same in outline.

Fig. 21.—Per. capensis. Gastrula stage, ventral view. Same stage as Fig. 19, but embryo slightly more elongated.

Fig. 22.—Per. capensis. Stage A, showing slightly elongated blastopore with primitive streak at hind end. Greatest length ·48 mm. a. Denotes the anterior end.
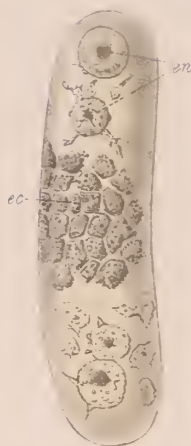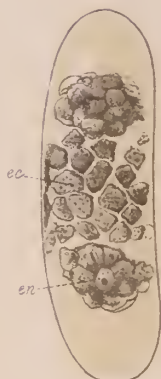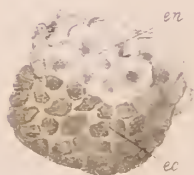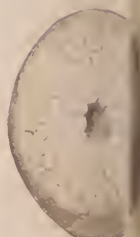
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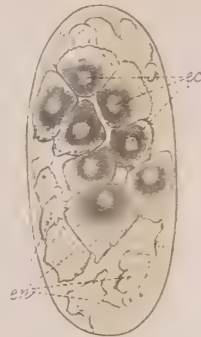
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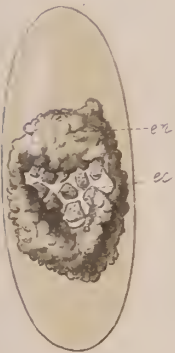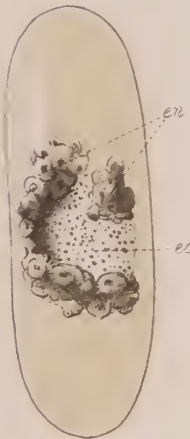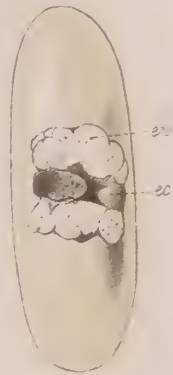
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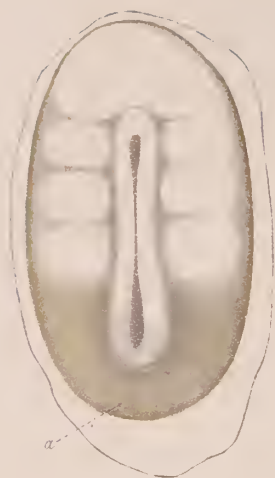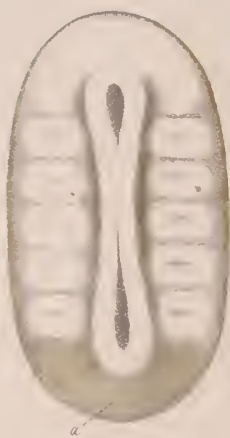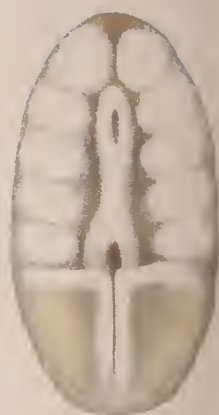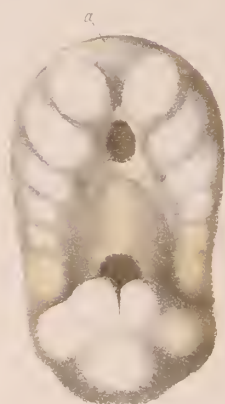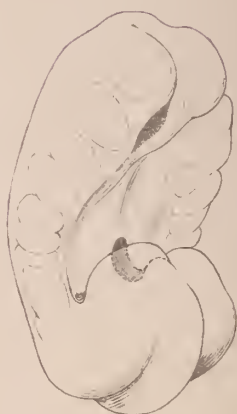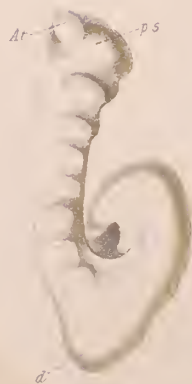
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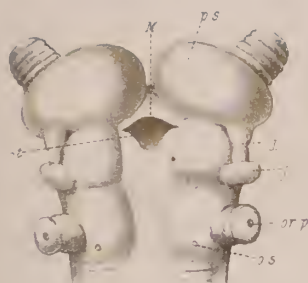
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PLATE XXXII.

All the figures on this Plate are of embryos of Peripatus capensis.

FIGS. 23—27.—From the original drawings by Miss Balfour. *a*. Denotes the anterior extremity.

The remainder of the figures on this Plate from drawings by Mr. E. Wilson.

Fig. 23. Stage between A and B. With three somites and elongated blastopore. Length of embryo ·7 mm., length of blastopore ·45 mm.

Fig. 24. Stage between A and B. With five somites. The blastopore is closing in its middle portion. Length of embryo ·74 mm., of blastopore ·46 mm.
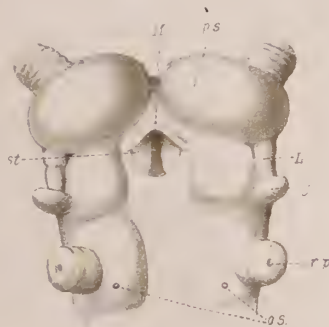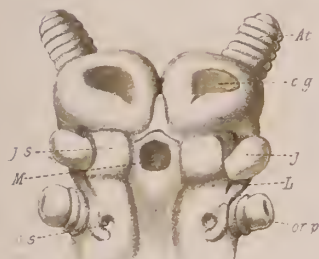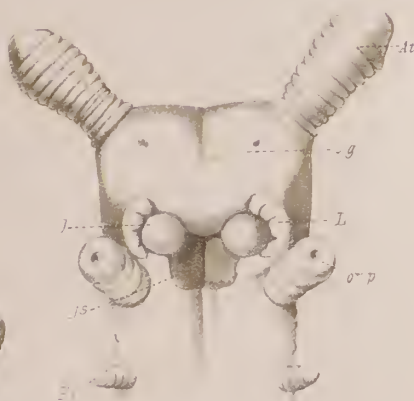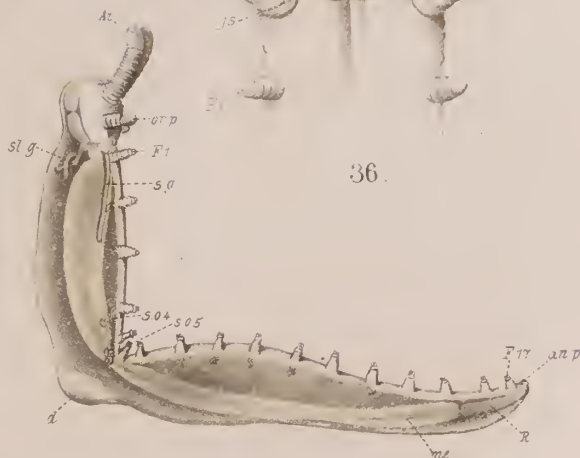
Fig. 25. **Stage B.** The blastopore has completely closed in its middle portion and given rise to two openings, the embryonic mouth and anus. The anterior pair of somites have moved to the front end of the body, and the primitive groove is very marked. Length of embryo 1·32 mm.

Fig. 26. **Stage C.** Embryo, in which the flexure of the hind end of the body has begun; with about thirteen somites. The remains of the original blastopore are present as the mouth, placed between the second pair of mesoblastic somites, and the anus, placed on the concavity of the commencing tail flexure, and still removed from the hind end of the body. Greatest length when lying on its back 1·12 mm.

Fig. 27. Side view of same embryo.

FIGS. 28 and 29.—Stage D (spiral stage).

Fig. 28. A young embryo of this stage, viewed from the side. With commencing antennæ and dorsal projection (*d*).

Fig. 29. Rather older embryo of same stage (end of spiral stage), side view. Eyes (*e*) as pits. Jaws and postoral appendages sprouting. Rudiments of eleven pairs of legs. Length from anterior end of head to bend (*d*) 1·6 mm. *At*. Antennæ. *d*. Dorsal thickening. *e*. Eye. *j*. Jaw. *or. p*. Oral papilla. *p. s*. Præoral somite.

FIGS. 30—34.—Stage E.

Fig. 30. Side view of straightened-out embryo. Antennæ ringed. Bucca fold (*L*) extending round the jaw (*j*) on to the ventral surface. Sixteen pairs of legs. Præoral somite ventrally grooved. Apex of oral papilla perforated. Length from front end of præoral lobe to bend (*d*) 1·76 mm. Eye still as an open pit (it is usually closed at this stage). *d*. Dorsal ectodermal thickening. *e*. Eye. *F*. 1 . . . . *&c*. Feet. *j*. Jaw. *L*. Lip. *or. p*. Oral papilla. *p. s*. Præoral somite.

Fig. 31. Embryo of same stage, in natural position in egg membrane.

Fig. 32. Hind end of embryo of same stage, ventral view. *a*. Anus. *s*. 20. 20th somite. *s*. 19 and 18. Legs of 19th and 18th somites.

Fig. 33. Ventral view of head and segments of jaws and oral papillæ of young embryo of same stage (E). Brain ungrooved. Ectoderm of sides of mouth beginning to grow inwards (*st.*). Oral papillæ perforated. Opening of salivary glands (*o. s.*) at base of oral papillæ. *j.* Jaw. *L.* Lips (buccal fold). *M.* Mouth. *o. s.* Opening of salivary gland. *p. s.* præoral somite. *st.* Ectodermal ingrowth at sides of posterior part of mouth to form stomodæum.

Fig. 34. Same view of slightly older embryo, with sides of mouth quite infolded (*st.*) and a new posterior border to the mouth formed. Brain still ungrooved. References as in Fig. 33.

Fig. 35.—Ventral view of head of embryo intermediate between Stages E and F. Grooves in brain wide and shallow. The lips have grown considerably and have extended behind the openings of the salivary glands, but have not yet joined in the middle line. *At.* Antennæ. *c. g.* Groove in brain. *j.* Jaws. *j. s.* Swelling at base of jaws. *L.* Lips. *M.* Mouth. *or. p.* Oral papillæ *o. s.* Opening of salivary glands.

Fig. 36.—Stage F. Groove in brain almost closed; the opening is slightly wider anteriorly. Lips complete and folded, salivary opening quite covered by them. Jaws completely enclosed. Swellings at base of jaws closely approximated so as to reduce the mouth opening to a narrow slit. The præoral lobes have completely united with one another (cf. Figs. 33—35). References as in Fig. 35.

Figs. 37 and 38.—Stage G.

Fig. 37. Side view of embryo of Stage G. Full number of legs and oral papillæ. Length 5—6 mm. *An. p.* Anal papillæ. *At.* Antennæ. *d.* Dorsal ectodermal thickening. *F.* 1—17. The legs. *me.* Mesenteron. *or. p.* Oral papillæ. *R.* Rectum. *s. g.* Salivary glands. *s. o.* 4 and 5. Segmental organs of 4th and 5th legs. *sl. g.* Slime glands.

Fig. 38. Embryo of Stage G, curled up as in the uterus.

On the Chromatology of the Blood of Some Invertebrates.

By

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With Plates XXXIII and XXXIV.

THE following account of a few observations made by me during the last two years on the blood of some invertebrate animals may prove of use to others engaged in the same kind of work, and although the observations are not by any means complete, I have thought it advisable to publish the results, with the remark that the present account is merely a preliminary one, and that I hope to follow up the subject more fully when an opportunity occurs of doing so.

As is well known, the colour of the blood in invertebrate animals does not as a rule belong to the corpuscles, but to what in them answers to the liquor sanguinis of Vertebrates, although there are many exceptions. In some hæmoglobin occurs. Thus, Prof. Lankester has shown[1] that in Glycera, Capitella and Phoronis, and in Solen legumen, it is found in special corpuscles; while in the vascular fluid of others it is found dissolved, e. g. with certain exceptions in some Chætopod Annelids, in some Leeches (Nephelis, Hirudo), in Polia sanguirubra (a Turbellarian), in the special vascular system of a marine parasitic Crustacean observed by E. van Beneden, in the general blood-system of the larva of the midge (Chironomus), in the general blood-system of the Mollusk Planorbis, and in the general blood-system of the Crustaceans Daphnia and

[1] " A Contribution to the Knowledge of Hæmoglobin," 'Proc. Roy. Soc.,' vol. xxi (1872), p. 71, &c.

Cheirocephalus.[1] Mr. Sorby has been inclined to doubt the exact identity in position of the bands of hæmoglobin in the blood of Planorbis with those of vertebrate hæmoglobin, but Prof. Gamgee shows that the bands there occur in the same position as those of vertebrate hæmoglobin—a statement which I can confirm.[2]

Hæmocyanin.—The blood of many Mollusks and Arthropods is of a blue colour after exposure to the air, and this is in most cases due to the presence of another pigment—hæmocyanin. This pigment has quite an extensive literature of its own. It is not within the scope of this paper to refer to all the work that has been done with regard to the latter colouring matter, so I will dismiss the matter in as few words as possible. In 1816 Ermann had observed the blue colour of the blood of Helix, which he thought was due to opalescence;[3] E. Witting[4] observed the feebly bluish blood of Unio pictorum, also that of Astacus, but he missed the blue colour in the latter case. Genth[5] observed the blue colour of the blood of Limulus cyclops in 1852. Rouget in 1859 made some observations on the blood of Octopus vulgaris (also on that of Sipunculus oxyurus[6]). In 1847 Harless and von Bibra observed the blue colour which the blood of Helix pomatia acquired on exposure to air and lost on treatment with CO_2; they also observed that ammonia removed the blue colour, which came back on neutralising with hydrochloric acid.[7] They stated that this blood contained copper, but no

[1] Lumbricus and Arenicola also contain hæmoglobin in their blood. Also Eunice.

[2] 'Physiological Chemistry,' vol. i, 1880, pp. 130, 131. Dr. Mays has obtained hæmin crystals from this blood. Cf. Krukenberg, loc. cit.

[3] "Wahrnehmungen über das Blut einiger Mollusken," 'Abhandl. d. K. Akad. d. Wissen. zu Berlin aus den Jahren 1816, 1817' (Berlin, 1819), S. 199—218.

[4] "Ueber das Blut einiger Crustaceen und Mollusken," 'Journ. f. pract. Chemie,' Bd. lxxiii, 1858, S. 121—132.

[5] "Ueber die Aschen bestandtheile des Blutes von Limulus Cyclops (Fabr.)," 'Ann. d. Chem. u. Pharm.,' Bd. lxxxi, 1852, S. 68—73.

[6] 'Journ. d. la Physiol.,' t. ii, 1859, pp. 660—670.

[7] 'Müller's Archiv,' 1847, pp. 148—157.

iron, but Gorup-Besanez found iron also in its ash.[1] Harless made an elementary analysis of the colouring matter, and found in it—besides copper—carbon, hydrogen, oxygen, and nitrogen. The same investigators examined the blood of the Cephalopods Loligo and Eledone; they found copper, but no iron, and stated that the blue colouration of the blood was removed by oxygen and restored on its abstraction—an error which has since been refuted.

In 1857 Haeckel[2] made some observations on the blood of Homola Cuvieri, in which he showed that the colourless blood became gradually grey and then black; he also observed that the bright blue blood of a lobster became after many hours a darker violet. P. Bert[3] in 1867 found the blood of Sepia colourless, feebly bluish, especially in the veins of the gills, and that it acquired a bright blue colour on exposure to air. This colour (he showed) belongs to the plasma, and is not lost by boiling. Rabuteau and Papillon[4] in 1873 experimented on the blood of the Octopus. They examined its spectrum, and arrived at the conclusion that it gives no bands; they found that it became blue on exposure to the air, that this colour was lost on treatment with CO_2, but on shaking with air again appeared. They observed the same colour changes in the blood of Crabs. Jolyet and Regnard[5] showed in 1877 that on shaking Crabs' blood with air it showed a beautiful blue or brownish colour according to the manner in which it was examined; it gradually loses this colour, becoming reddish and then feebly yellow, but on treatment with pure oxygen its original colour is restored. They found two colouring matters[6] in Crabs' blood; one is blue, and is precipitated by alcohol with the albumen of the blood; the other is reddish, and remains in the alcoholic filtrate.

[1] 'Lehrbuch d. physiol. Chemie,' p. 369.
[2] Müller's Archiv,' 1857, S. 511, Anm. i.
[3] 'Compt. rend.,' t. lxv, 1867, pp. 300—302.
[4] 'Compt. rend.,' t. lxxvii, 1873, p. 137.
[5] 'Extr. des Archives de Physiologie,' 2 sér., t. iv, 1877, p. 36, &c.
[6] See p. 475, foot note.

In 1878 Fredericq,[1] in a paper on the physiology of Octopus vulgaris, showed that the blue colouring matter of the blood of this species is combined with a proteid and with copper; the proteid is more complex than an ordinary proteid, since it yields one as a decomposition product. Fredericq found that this blood lost its colour in vacuo and regained it on treatment with oxygen; and he observed its blue colour in the arteries of the living Octopus. He found that it showed no absorption bands. The following year Fredericq[2] stated that the colouring matter of the blood of the lobster is identical with that of Octopus; it behaves in the same manner in vacuo and on treatment with oxygen; he further showed that the statements of Jolyet and Regnard with regard to Crabs' blood also apply to the blood of the lobster, which is blue with reflected and brownish with transmitted light. The red pigment also present does not belong to the albuminous constituents; it contains no copper, and has nothing to do with the change of colour of the blood, nor is it constantly present in the blood. The same authority showed that the blood of Helix and Arion contains a similar blue colouring matter to that of Octopus, Homarus, and Crabs, i. e. hæmocyanin. Prof. Lankester[3] has shown that the blood of Limulus and Scorpio becomes blue on exposure to air, and contains hæmocyanin; and Golch and Laws,[4] of Oxford, have found that in Limulus polyphemus the blood colouring matter was allied to hæmocyanin, and contained a proteid united to copper, like it.

Krukenberg[5] has examined the blood of Eledone moschata, Sepia officinalis, Homarus vulgaris, Carcinus maenas, Eriphia spinifrons, Portunus depurator, Grapsus

[1] 'Extr. des Bulletins de l'Acad. r. de Belgique,' 2 sér., t. xlvi, No. 11, 1878, pp. 4—21.

[2] 'Extr. des Bulletins de l'Acad. r. de Belgique,' 2 sér., t. xlvii, No. 4, Avril, 1879.

[3] 'Quart. Journ. Micro. Sci.,' 1878, p. 453, et seq.; ibid., vol. xxiv, p. 151.

[4] 'Brit. Assoc. Reports,' 1884.

[5] 'Vergleich. physiol. Studien.,' 1st Reihe, 3 Abth., 1880, S. 72, &c. The above account of the literature of hæmocyanin is partly taken from this work. I am also indebted to Gamgee's 'Physiological Chemistry' for a few references.

marmoratus, Maja verrucosa, Pilumnus villosus, Squilla mantis, and in all has seen the blood become blue by shaking with air and oxygen, and the blue colour disappear more or less with CO_2. He thinks it probable that a part of the hæmocyanin or of its uncoloured reduction product becomes decomposed or insoluble in the blood after some hours' quiet standing. In Limnæus stagnalis he found that the blood after becoming blue on exposure to air was hardly changed in colour on shaking with CO_2; now, it seems to me that a current of CO_2 ought to be conducted into the blood before one can arrive at a negative conclusion. Krukenberg, however, thinks that in the blood of Helix pomatia and aspersa, and of Limnæus stagnalis a body exists which is at least very nearly related to hæmocyanin. Perhaps he might have gone further, and concluded that hæmocyanin is present, as Fredericq has shown for the first species to be the case. Krukenberg also found great differences in the blood of individual Gasteropod Mollusks, which led him to assume that perhaps the oxygen in such cases is in a firmer combination with the hæmocyanin than is the case in Crabs and Cephalopods. He also made the interesting observation that the blood of Crabs and Cephalopods on treatment with carbonic oxide became colourless, but regained its blue colour on shaking with air. This behaviour is different from that of hæmoglobin when similarly treated. It was further found that blood which had become blue by reception of oxygen if allowed to stand in a test-tube exposed to the air did not lose its blue colour from above downwards, but from below upwards, whence he concludes that the blueing is not due to suspended particles, but to the presence of a chromogen which becomes blue by reception of oxygen. With H_2S the blue Crabs' and Eledone's blood became a feeble yellow, and lost the property of again becoming blue with oxygen. He could find no hæmocyanin in the blood of several Mollusks (e. g. Tethys fimbria, Doris tuberculata, Aplysia depilans, Pleurobranchus, &c.[1]).

[1] Krukenberg further shows that (besides Planorbis in which the blood is

In Anodonta cygnea C. Schmidt[1] found the blood colour-
less. My own observations on the blood of Mollusks and
Arthropods have been scanty, and made to determine whether
absorption bands are present or not. I have examined the
blood of Helix pomatia, Helix aspersa, Paludina vivi-
pera, Limnæus stagnalis, Homarus vulgaris, Cancer
pagurus, Carcinus mænas, and Astacus fluviatilis, but
in none of them could I see any bands.

The blood of Helix aspersa was found to be a bluish-
white colour by daylight, but by gaslight it had a purplish
tinge; after twenty-four hours' standing that had disappeared,
and it was then very slightly brownish. Examined in a deep
layer, no bands could be seen; on treatment with ammonia,
the blue colour persisted and no bands came into view. With
acetic acid the blue colour persisted, and no bands appeared.
After repeated filtering the blue colour remained, hence it
can hardly have been due to particles in suspension. On
treatment with reducing agents the blue colour was lost, and
no bands appeared.

Blood of Helix pomatia.—The blood assumed a distinct
blue tinge on exposure to the air, and gave no absorption
bands, but absorbed a little of the violet end of the spectrum.
On treatment with ammonia its colour was not so well
marked and it had a faintly reddish tinge, but no bands
could be seen, nor after treatment with acetic acid which did
not remove the colour. On treatment with sulphide of
ammonium the blue colour disappeared and could not be again
brought back by shaking with air, the solution being free
from bands. In some specimens exposed to the air for some
time the fluid had assumed a bronze colour, and with gaslight
a faint violet tint, but no bands were seen.

red) the blood of Apus is intensely red (it contains hæmoglobin like that of
its congener Cheirocephalus, as shown by Lankester), of Gammarus violet
(v. Siebold), of Limnadia ruby red (Klunzinger), of Palinurus (Lund and
Schultz) and Astacus (Haeckel) pale red, of Lernanthropus (C. v. Heider)
reddish-yellow.

[1] Lehmann's 'Physiol. Chem.,' vol. iii, p. 256.

Blood of Limnæus stagnalis.—On exposure to air it assumed a whitish-blue colour, gave no bands, nor after treatment with ammonia, acetic acid, or sulphide of ammonium; the last discharged the colour completely, which could not be restored on shaking with air.

Blood of Paludina vivipara.—The blood of this Mollusk is frequently exuded when the animal is pricked with a needle or otherwise irritated, and is of a blue colour. It is quite free from bands. Ammonia slightly diminishes the colour, but does not remove it; acetic acid does not remove it; with neither reagent nor with sulphide of ammonium could any distinct bands be obtained.

The blood of Homarus, Cancer, Carcinus, and Astacus[1] were also examined with the same negative result as regards bands, their colouring matters are, I believe, all identical, and generally agree when present with the description of hæmocyanin given by Fredericq and others referred to above. I need not therefore enter into further detail as this paper deals only with the spectroscopic characters of the colouring matters.

The blood of Serpula contortuplicata and Sabella tubularia (Gosse). — A few preliminary remarks on the chromatology of the blood of some worms is necessary before describing the results of my examination of the above. Those worms which contain hæmoglobin in their pseudohæmal system or perivisceral cavity have already been referred to.

In his paper above referred to Professor Lankester mentions the fact that Sipunculus nudus of the Gulf of Naples contains a pale madder-like colouring matter in its perivisceral cavity, which is due to a large number of coloured corpuscles from $\frac{1}{3500}$th to $\frac{1}{4000}$th of an inch in diameter, and that this colouring matter, also found in other parts of the worm, is not hæmoglobin.

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[1] Halliburton has shown since the above was written that in the blood plasma of Homarus a red pigment, soluble in alcohol, ether, chloroform, &c., occurs, which appears to be the same red pigment as that found by me in other parts of the same animal. See his report on Proteids of Blood, ' Brit Med. Journ.,' July 25th, 1885.

Delle Chiaje[1] showed that in S i p u n c u l u s b a l a n o r o p h u s and e c h i n o r h y n c h u s the arterial blood is red, the venous brown.   G. Schwalbe[2] found that the body-fluid of P h a s c o-l o s o m a e l o n g a t u m (a Gephyrean) is a bright rose- or greyish-red colour, and is cloudy owing to the presence of morphological elements, and that on standing in the air it gets darker and darker until it assumes an intense Burgundy-red colour.   By long standing in the air this colour goes into a dirty brown owing to decomposition, and in drying the whole assumes a dirty green colour.   Krukenberg[3] found the blood of S i-p u n c u l u s n u d u s to contain the same colouring matter as that observed by Schwalbe ; he finds that it is the oxygen of the air which brings about the colour change, and that the colour is removed by CO_2. This colouring matter gives no absorption band either in the oxidised or reduced condition.   Krukenberg calls this pigment h æ m e r y t h r i n, and the chromogen belonging to it h æ m e r y t h r o g e n.   The colouring matter is decomposed by H_2S.   The oxygen in the oxidised blood-pigment seems, according to Krukenberg, to be more firmly fixed than in oxy-hæmoglobin.   Milne-Edwards[4] in 1838 discovered that certain Annelids possessed green blood, his observations being made on S a b e l l a.   In C h l o r o n e m a E d w a r d s i M. de Quatrefages found similar blood.

Professor Lankester[5] on examining the blood of S a b e l l a v e n t i l a b r u m and S i p h o n o s t o m a (sp. ?) with the spectro-scope discovered the interesting fact that it not only gives a banded absorption spectrum, but is capable of being oxi-dised and reduced, and it behaved in such a way with cyanide of potassium and sulphide of ammonium as to have led him to conclude that hæmoglobin and this colouring matter (which Pro-fessor Lankester named chlorocruorin) " have a common base

[1] 'Memorie sulla storia e notomia degli animali senza vetebre del regno di Napoli,' t. i, pp. 13 and 127.

[2] 'Arch. f. Mikr. Anat.,' Bd. v, p. 248, et seq., 1869.

[3] Loc. cit., p. 85.

[4] 'Ann. des Sciences Natur.,' 1838, 2nd série, vol. x, p. 190.

[5] 'Journ. of Anat. and Physiol.,' 1868, p. 114 ; also 1870, p. 119.

in cyanosulphæm, and perhaps in Stokes' reduced hæmatin."
Now, Krukenberg[1] tries to show that this reduction is not such,
but that chlorocruorin and erythrocruorin are one and the
same substance which is not reduced by sulphide of ammonium.
If Krukenberg had studied Professor Lankester's paper he would
have seen that Professor Lankester says that "on addition
of reducing agents the two bands are changed into one, having
nearly the same position as the darker of the two but fainter.
On agitation with air the two returned." The extra-
ordinary mistake of Krukenberg in missing the fact that
what he calls "Helicorubin"—and takes to himself the credit
of having discovered, although previously discovered by Dr.
Sorby—is capable of existing in the oxidised and reduced
state, is an exact parallel to this.[2]

His other criticisms on the action of cyanide of potassium
on chlorocruorin are based on a mere comparison of spectrum
maps and are therefore valueless.

Professor Lankester could not obtain derivatives of chloro-
cruorin, owing, as he has stated, to the apparent instability of
this body, which decomposes rapidly.

I have really nothing of importance to add to the descrip-
tion given by Professor Lankester, but I have examined the
aqueous solution of chlorocruorin, its behaviour with some
reagents, and measured its bands in wave lengths. For the
sake of comparison I have mapped these spectra in the accom-
panying Chart I. I have only been able to obtain about a
dozen specimens, so that my examination is not complete, but
I hope to be able to study this subject again.

On slitting up a worm and collecting the green fluid which
exudes on a watch-glass and examining with the microspec-
troscope a dark band is seen before D, and a feeble one
between D and E (sp. 1, Chart I). The brown gills of the

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[1] Loc. cit. and 'Grundzüge einer Verlgleichenden Physiologie der Farb-
stoffe und der Farben,' 1884.

[2] I have shown that this gives the bands of hæmochromogen. Kruken-
berg's map of it is quite incorrect. See my sketch in 'Proc. Roy. Soc.,' No.
226, 1883. I have lately proposed the name "enterohæmatin" for this
pigment.

specimens examined by me gave no bands, and did not contain therefore the same pigment which I found in those of Serpula to be referred to further on.

The green fluid had a reddish tinge with reflected gaslight, and in most cases was green with transmitted daylight, and reddish with transmitted gaslight. On dilution with water the fluid gave two bands :—the first from λ 618 to λ 593, the second from λ 576 to λ 554·5. On then adding ammonium sulphide I obtained sp. 2, Chart I. As well as I could make out the first of these bands extended from λ 625 to λ 596·5 (?), but this and also the second band were very faint. If now caustic soda were added to this solution a dark band was seen covering D, which recalls to mind the band of alkaline hæmatin (sp. 3, Chart I), and this band extended from λ 595 to λ 576.

If an aqueous solution is treated with caustic soda alone this appearance is not seen, as the bands become faint and gradually disappear, but if then ammonium sulphide is added, the same band covering D appears (sp. 3, Chart I).

If the blood is treated with rectified spirit and caustic potash and filtered a yellowish solution is obtained free from bands, but on adding ammonium sulphide a band appears covering D, as in the case of the aqueous solution (sp. 4, Chart I).

In some specimens the second band did not occupy the same position as before in aqueous solutions of the blood ; it sometimes read from λ 569 to λ 551, and with ammonium sulphide the band of this reduced chlorocruorin extended from λ 623 to λ 593 (see sp. 5 and 6). But on adding caustic soda to this reduced fluid the band, like that of sp. 3, again appeared.

On treating aqueous solutions with acetic acid the bands faded away, and the colour of the solution changed to brownish (gaslight).

I tried the action of rectified spirit acidulated with sulphuric acid on chlorocruorin, and obtained a faint greenish solution, which showed a faint shading in the green too indistinct to map.

On treatment with sulphuric acid a brownish-yellow solution

was obtained, which, on adding absolute alcohol, showed a band in green, but as it is very doubtful whether this is not due to the action of the acid on a proteid I have not mapped the spectrum.

Hence none of the decomposition products of hæmoglobin or hæmatin could be obtained,[1] the pigment, as Prof. Lankester had already shown, being destroyed by the reagents required to produce acid hæmatin and hæmatoporphyrin. I digested the gills of several specimens of Sabella in chloroform, but failed to obtain a coloured solution ; digestion with rectified spirit and caustic potash furnished a yellow solution, but in this no well-marked bands could be detected.

The blood[2] of the pseudo-hæmal system of Serpula con-tortuplicata presents some resemblance to that of Sabella, and I believe it has not been examined until now. There are slight differences in the blood spectra of some specimens, which doubtless are due to the pigment being present in different states of oxidation, and on comparing some of these spectra with those of the histohæmatins and with decomposition pro-ducts of hæmoglobin a striking likeness is apparent.

On putting a Serpula into the compressorium, and bringing gentle pressure to bear on the upper surface of the animal, and examining with the microspectroscope, using a good achromatic substage condenser, a series of spectra are obtained when the various parts of the animal are moved under the objective ; what these parts are is seen by looking down the left-hand tube of the microscope.

In this way we can differentiate the blood-vessels, intestine, gills, operculum, and other parts, and study the spectrum of each. A portion of the pseudo-hæmal system, with its con-tained blood of a worm gave sp. 7, Chart I, the band before D is like that of chlorocruorin, but the first after D and also the

[1] At the same time this colouring matter is certainly closely related to hæmatin, as sps. 2 and 8 show. In 8 (from serpula-blood) bands like those of hæmochromogen are present.

[2] I have not given this pigment a name, as I believe it to be a kind of chlorocruorin.

second are different. In some of the blood-vessels only the band before D was visible.

An aqueous solution obtained from nine Serpulæ was a reddish-yellow colour by gaslight, yellow by daylight, and this gave a spectrum like 7. The band before D was from λ 620·5 to λ 593, the second about λ 583·5 to λ 572, the third uncertain (about λ 551 to λ 532). After adding sulphide of ammonium the only band seen with certainty was that before D. which seemed slightly nearer violet. In rectified spirit extracts only a faint lutein band was visible in the yellow solution from about λ 501 to λ 477.

In a specimen in which the blood appeared a bright carmine-red colour sp. 8, Chart I, was obtained; the second band of this spectrum resembles the first band of hæmochromogen, and is really the same as sp. 2. The principal blood-vessel showed two round dilatations, and in these I observed sp. 9, Chart I. The darkness of the second band at once distinguishes the pigment from chlorocruorin.

In other specimens the same dilated part of the pseudo-hæmal vessels gave sp. 10, Chart I, while in another specimen the blood showed sp. 11, Chart I.

An aqueous solution of blood obtained from a dozen specimens—whose blood gave the above spectra—was yellow, and showed the three bands represented in sp. 12, Chart I, and these gave the following readings :—First band λ 618 to λ 593,[1] second λ 582 to λ 570·5, third λ 551 to λ 529·5 (?). On treatment with sulphide of ammonium the solution became slightly greener ; no bands could then be seen after D, and that before it was very faint. Hence it would appear that the two- or three-banded spectrum denotes the oxidised state.

On digesting the bodies of some S e r p u l æ in caustic potash and rectified spirit a yellow solution was obtained, giving no definable bands, except some feeble shading at the blue end of green, but on adding sulphide of ammonium the solution became faintly red, and a band like the first band of

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[1] I.e., exactly the reading of the first band of oxychlorocruorin.

hæmochromogen was just visible, and perhaps a second like its second band.

In some Serpulæ whose blood was not red but brown, the bands before and after D reminded of chlorocruorin (sp. 13, Chart I). In these, too, the gills were not red, as in the other specimens, and failed to show a band. An aqueous solution of the blood of these specimens had a reddish tint by gaslight, and gave three bands, which read as follows :—First λ 620·5 to λ 595, second λ 583·5 to λ 570·5, third λ 551 to λ 532. On adding sulphide of ammonium the band before D read λ 620·5 to λ 598, and a second band was visible after D, which could not be measured. On adding to this reduced fluid some caustic soda at first the only change produced was the disappearance of the faint band after D, but, after standing, sp. 14, Chart I, appeared, of which the bands read: first λ 623 to λ 607, second λ 596·5 to λ 579. This shows that the blood of these Serpulæ did not contain the same kind of chlorocruorin as Sabella, but a pigment very closely related to it, probably nearer to hæmatin than it. I had not enough material for further study.

In most cases the gills gave sp. 15, Chart I, while in others the band was slightly nearer violet. On extracting them with absolute alcohol an orange solution was obtained, which strongly absorbed the violet end of the spectrum, and allowing only the red and beginning of the green to pass in a deep layer. In a shallow one a shading was seen from about λ 509 to λ 467 (?). This solution became blue, and then colourless with nitric acid, but was not much changed by hydrochloric acid. Caustic potash developed a more distinct shading, from λ 509 to λ 481.

The red opercula gave a band covering D, sp. 16, Chart I; they vary much in colour, some being very red, while others are colourless, and in some gills the same band is seen.

The band of sp. 15, Chart I, belonging to the gills, though resembling that of reduced hæmoglobin, has no connection with it, as I found by extracting the gills with rectified spirit and caustic potash, and adding sulphide of ammonium, the

result obtained being negative, so far as hæmochromogen is concerned. The pigment present in them is closely related to, if not identical with, tetronerythrin, and in the hypoderm of Cancer pagurus and integument of Uraster rubens, where I have shown tetronerythrin[1] to be present, the solid pigment gives the same kind of band. In some Serpulæ I could perceive the bands of the colouring matter of the blood itself in the gills. The tetronerythrin of the gills and other red parts is probably of no respiratory use, and I think its action with reducing agents in other cases goes to show that too much importance has been attached to its supposed respiratory functions. It is not unlikely that, especially when its likeness to Kühne's chromophanes is taken into consideration, it may be of use in absorbing certain rays of light concerned in some obscure photochemical process.

The colouring matter of the perivisceral fluid of Strongylocentrotus lividus. In 1883[2] I stated that in various parts of the body and in the perivisceral fluid of Echinus (esculentus?) and sphaera I had detected a colouring matter of a brown colour which gave two bands, one between D and E covering E, the other between b and F, the first of which became decidedly darker with ammonium sulphide. I found that it went into chloroform and alcohol, but owing to a dearth of material I did not arrive at any very definite conclusions, except that this pigment is respiratory. Professor Schäfer informed me some time after the publication of these results that in working at the coagulation of the perivisceral fluid of an Echinus he had seen the same bands; and P. Geddes[3] had observed the colour changes of a similar pigment. The latter observer has worked out the morphology of the corpuscles of the perivisceral fluid of various Echinoderms[4] and

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[1] 'Proc. Birm. Philos. Soc.,' vol. iii, 1883.
[2] 'Proc. Birm. Philos. Soc.,' loc. cit., pp. 380, 381.
[3] See Gamgee's 'Physiological Chemistry,' pp. 134, 135.
[4] 'Proc. Roy. Soc.,' No. 202, 1880. I have not seen his paper 'Archives de Zoologie Expérimentale.'

has made observations on its coagulation; he does not, however, say anything about its spectroscopic characters.

I named this pigment echinochrome and have lately made several observations on fresh specimens of Strongylocentrotus lividus, which I was able to procure alive in considerable quantity. On opening a specimen a fluid of a pale red colour exudes from the perivisceral cavity; it sometimes has a violet tinge. In a short time a clot forms; this becomes gradually darker in colour and it contracts more and more, until all its connections with the side of the containing vessel are broken, and it finally shrinks into a small brown-red mass. The corpuscles are carried down by this clot and it is to them, not to the plasma, that the colouring matter belongs. Geddes[1] has shown that the colourless, finely granular, pale corpuscles run together to form plasmodia, and that it is to their fusion that the clotting is due.

The corpuscles present all degrees of colouration, from a brilliant lake red, through a pale orange, to colourless. The red ones are nucleated and of irregular shape, and rapidly throw out amœboid processes, so also do the others. The nucleus is strongly refracting and gives the corpuscle the appearance of a round hole having been punched in it. The red corpuscles measure from $\frac{1}{1500}$th inch in long diameter $\times$ $\frac{1}{3000}$th in short, down to $\frac{1}{3000}$th in long $\times$ $\frac{1}{6000}$th in short, while several measure $\frac{1}{3000}$th in both diameters. The pale ones $\frac{1}{1500}$th $\times$ $\frac{1}{2000}$th down to $\frac{1}{3000}$th $\times$ $\frac{1}{6000}$th; the latter are multi-nucleated.[2]

The pigment itself in the fresh state showed no distinct bands but treated with caustic potash in the solid condition the colour changed to dark purple and showed the bands of sp. 1, Chart II.

It seems to me that the deepening of colour which echino-chrome undergoes on exposure to the air must be in part due to the oxidation of a chromogen, if so we may infer the existence of such, and name it echinochromogen.

[1] Loc. cit.

[2] I think the red corpuscles only differ from the white in possessing pigment.

It is not a difficult matter to obtain solutions of this colour-ing matter for spectroscopic examination, as it is taken up by a great number of solvents, in which point it differs much from the blood pigment of most invertebrate animals; and it resembles in its solubility lutein and tetronerythrin (= Kru-kenberg's lipochromes); still its spectroscopic and other cha-racters show that it is not either one or the other.

Echinochrome can be obtained in solution and isolated by two methods : (1) The fresh blood-clot can be extracted with the solvents mentioned below, or (2) the clot may be separated from the serum by filtering, the pigment dried at the tempera-ture of the air (as it changes by using heat) and the dried pigment thus obtained treated by solvents. By the adoption of the latter method it can be obtained in a purer condition.[1]

The "serum" after separation of the clot is a faint yellow colour and shows two faint bands in green, but if allowed to stand some time in contact with the clot it becomes a faint violet red, and then shows Chart II, sp. 2. Treated with caustic soda these bands are intensified, but then the fluid is not quite as coloured as before, but if instead stannous chloride be added the dark bands of sp. 3, Chart II, appear, the fluid being then violet-reddish. These bands read from λ 541·5 to λ 532 and λ 506 to 486·5; on agitation with air they are not as distinct but do not altogether disappear.[2] The serum was found to be faintly acid, or neutral, faintly opalescent on heating, opales-cent with absolute alcohol, and faintly so with ether.

The brownish-red clot shows after standing in contact with the "serum" sp. 4, Chart II, and with caustic soda sp. 5, II.

An absolute alcohol solution of the fresh clot is a red colour, allowing red, orange, yellow, and a little green to pass in a deep layer, while in a thinner layer examined by day-light sp. 6, Chart II is seen. These bands have the following

[1] The filter paper with the dried pigment on it is cut up and put into test-tubes containing the solvents, and corked up and left in a dark place.

[2] Because in the oxidised condition bands of the same kind, but feebler, are visible.

positions : 1st, λ 557 to λ 545·5,[1] 2nd, λ 524·5 to λ 501, 3rd, λ 494·5 to λ 475.   This third band is merged into the second. On adding sulphide of ammonium two new bands appear of which the 1st is from λ 531 to λ 507, and 2nd, λ 494·5 to λ 475, the colour of this solution being changed to yellow and on shaking with air remaining the same.   With caustic soda similar bands appeared and the solution became yellow ; the bands read : first, λ 532 to λ 509 ; and second, λ 494·5 to λ 477.   On neutralising this solution with acetic acid it became again faintly red, and the original bands reappeared but were very faint.

On treatment of an absolute alcohol solution with acetic acid the colour changed to reddish yellow and sp. 7, Chart II, was seen.   On treating with caustic soda to alkalinity, the same bands as those seen when an alcohol solution is treated with that reagent appeared.   The spectrum of the original absolute alcohol solution is that of the neutral pigment, as can be proved.   Peroxide of hydrogen did not affect the bands.   Hydrochloric acid produced the same effect as acetic acid ; the bands reading : first, λ 545·5 to λ 529·5 ; second, λ 511·5 to λ 488. When the alcohol solution is treated with stannous chloride the colour changes to yellow, and two very well-marked bands appear (sp. 8, Chart II).   Dark part of first band λ 535 to λ 511·5 ; second, λ 496·5 to λ 477.   Hyposulphite of sodium changed the colour to yellow but the original bands could be seen, although faint.

On evaporating the alcohol solution on the water-bath a brownish-red residue was left, in which were numerous crystals of chloride of sodium ; on treating with chloroform a fine red solution was obtained, but a good deal of the residue remained undissolved, and what did remain of it was more of a pink colour than its previous colour brown ;[2] on filtering the chloroform the paper was stained a pale rose colour.   On a white dish this chloroform solution had a violet tinge and gave sp. 9,

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[1] If the feeble shading at each side of the band is included it reads from λ 560 to λ 543.

[2] Owing to decomposition.

II ; on evaporation of the chloroform a brown-red residue with a violet tinge was left. This residue was now only partially soluble in absolute alcohol, forming a pale reddish-violet solution, giving, however, the same spectrum as the original absolute alcohol solution, and with caustic soda it gave the same reaction as before. From the fact, however, that the whole of the residue was not now soluble in absolute alcohol, it is quite evident that the pigment was decomposed by evaporating it down by the aid of heat. I stated above that the residue left after the evaporation of an alcohol solution was not quite soluble in chloroform, and what was left after the chloroform extraction was treated with ether; this solvent took up a pigment whose absorption spectrum is remarkable for the two narrow bands in green, which recall to mind some histohæmatin spectra. This ether solution was reddish, and gave sp. 10, Chart II ; its bands reading as follows: first band, λ 554·5 to λ 547; second, λ 540 to λ 535 (?) ; and third, about λ 516 to 484·5 (= darkest part). Although so different in spectrum from the alcohol solution, yet when the ether was evaporated and the residue again dissolved in absolute alcohol, a rose-coloured solution was obtained whose spectrum closely resembled that of the original alcohol solution, and with caustic soda it changed to yellow, and the bands already referred to were seen. Hence the pigment present in the ether could not have been much changed. After the extraction of the residue left after the evaporation of the absolute alcohol by ether and chloroform, some was still left untouched by these solvents ; on treatment of this residue with nitric acid the colour was discharged, the acid itself now becoming yellowish.

That the above ethereal solution contained a slightly different colouring matter from that present in chloroform is proved by this experiment. An alcohol solution of clot was evaporated down, the residue extracted with chloroform, the latter evaporated down ; it then left a brown amorphous pigment. On dissolving this in ether I obtained not sp. 10 but sp. 11; but when the residue from the alcohol solution already extracted by chloroform was treated with ether, a reddish-

yellow solution was obtained which did give sp. 10 again, showing the splitting up of the echinochrome into two pigments.

If a chloroformic solution obtained as above be evaporated and the residue[1] extracted with bisulphide of carbon, a red solution is obtained which gives sp. 12, Chart II. The residue is also soluble in benzene, giving similar bands.

If the second of the methods mentioned above for obtaining echinochrome—namely, filtering off the clot from the " serum," drying on the paper, and extracting with absolute alcohol—be adopted a pale red solution is obtained, which gives the same spectrum as the alcohol solution of fresh clot, and the same spectrum with stannous chloride.

An aqueous solution of dried echinochrome gave no distinct bands, but on adding stannous chloride the usual bands appear, which still persist after acidulating with hydrochloric acid; after which they read : first band, λ 537 to λ 513; second, λ 505 to 484·5 (?) ; the colour of the solution being reddish.

An ether solution of the dried clot is reddish-yellow and gives sp. 13, II ; that this solution contains the same pigment as the alcohol solution is shown by adding stannous chloride which develops the bands referred to, measuring in this case : first band, λ 533·5 to λ 520; second, λ 496·5 to λ 477, the solution having a violet-red tint. On treatment of this with an ether solution of peroxide of hydrogen the bands produced by the stannous chloride were not changed.

A chloroform solution of the dried clot is reddish yellow —and in one degree of dilution violet red—and gives sp. 14, Chart. II. On treating with stannous chloride the usual bands are seen : first, from λ 540 to λ 516 ; second, from λ 505 to λ 484·5 (?).

A bisulphide of carbon solution of the dried clot is violet red, and gives sp. 15, Chart II. The bands measure approximately, first λ 566 to λ 554·5, while the dark shading commences at λ 537, and its darkest part ends at λ 511·5, the

[1] Which is of a violet colour.

feeble shading extending to λ 484·5, and with stannous chloride the result was the same as before.

A benzene solution of the dried clot gives sp. 16, II, and is similarly changed by stannous chloride ; the bands produced by this reagent :—measuring first from λ 538·5 to λ 516, the second λ 505 to λ 484·5.

A petroleum ether solution gave a similar spectrum, and altered in the same manner by stannous chloride.

Glycerine is also a good solvent for fresh echinochrome, it forms with it a deep red solution in which two bands are seen, the first from λ 560 to λ 545·5, sp. 17, II. On treatment with caustic soda the solution is reddish-yellow, and two bands (as in other cases) are seen ; the first from λ 541·5 to λ 516, and the second λ 503 to 484·5 (?). On adding acetic acid to this solution they disappear, and they can be brought back with more caustic soda. When a glycerine solution is treated with acetic acid, the same change as that produced by this reagent in the case of alcohol solutions takes place (see Chart II, sp. 7). Hydrochloric, sulphuric, and nitric acid produce the same effect. Ammonia changes the red colour to orange yellow, and this solution shows two bands : first, λ 537 to λ 516 ; second, λ 501 to λ 482·5. On treating a glycerine solution with stannous chloride the solution is reddish yellow and gives sp. 18, II ; the first band is from λ 540 to λ 513, the second from λ 503 to λ 481 ; they are unaffected by hydrochloric acid. The above are the most important characters of echinochrome ; the colours of the solutions were observed mostly by gaslight owing to circumstances over which I had no control.

Although I have now examined a great number of animal colouring matters, I have not met any which—as regards spectra and solubility—resembles this one.

It is partially soluble in water and alcohol, soluble in gly-cerine, ether, chloroform, benzene, bisulphide of carbon, and petroleum ether. It is certainly capable of existing in two states of oxidation,[1] and is therefore respiratory. It is—when

[1] The surmise of Krukenberg that the appearance of the dark bands on

its solutions are evaporated—quite amorphous, as in no instance have I been able to obtain it crystallised.

The spectroscopic study of the blood of Ascidians and some other Invertebrates I hope to continue shortly, but my observations in these cases are not sufficiently advanced to allow of their being published at present.

EXPLANATION OF PLATES XXXIII & XXXIV,

Illustrating Dr. C. A. Mac Munn's Paper "On the Chromatology of the Blood of some Invertebrates."

CHART I.

Sp. 1.—Spectrum of Professor Lankester's oxychlorocruorin, from the green fresh blood of Sabella.

Sp. 2.—The same with ammonium sulphide, after dilution with water. (Note the attempt at the first band of hæmochromogen.)

Sp. 3.—The solution treated with caustic soda, after ammonium sulphide; the band recalls to mind that of alkaline hæmatin.

Sp. 4.—The blood is treated with rectified spirit and caustic potash, and then treated with sulphide of ammonium.

Sp. 5.—Aqueous solution of oxychlorocruorin.

Sp. 6.—The same with ammonium sulphide.

Sp. 7.—Spectrum of the blood (while in the living animal) of Serpula contortuplicata.

Sp. 8.—Spectrum of the bright carmine-coloured blood of another worm. (Note the bands like those of hæmochromogen.)

Sp. 9.—Ditto of a dilatation of the principal pseudohæmal vessel of a Serpula.

Sp. 10.—Ditto in another specimen.

Sp. 11.—Spectrum of the blood of another specimen, evidently like that of oxychlorocruorin.

Sp. 12.—Aqueous solution of blood of a Serpula.

Sp. 13.—Brownish blood of another specimen.

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adding reducing agents is caused by precipitation, is sufficiently refuted by what I have shown in this paper.

Sp. 14.—The same blood in aqueous solution after the addition of sulphide of ammonium and caustic soda.   Compare Sp. 3.

Sp. 15.—Spectrum[1] of the gills of some Serpulæ.

Sp. 16.—Spectrum of an operculum.   In the gills of some Serpulæ this band is seen.

CHART II.

Sp. 1.—The colouring matter of the perivisceral fluid of Strongylocentrotus lividus with caustic potash (in the solid state).

Sp. 2.—The "serum" of this fluid, after standing in contact with the clot for a considerable time.

Sp. 3.—Action of stannous chloride on the same.   Reduced echinochrome.

Sp. 4.—Spectrum of the brownish clot (of same fluid).

Sp. 5.—The same treated with caustic soda.

Sp. 6.—Absolute alcohol extract of fresh clot.

Sp. 7.—The same with acetic acid (acid echinochrome).

Sp. 8.—Absolute alcohol extract of fresh clot with stannous chloride.

Sp. 9.—Chloroform extract of residue from the evaporation of an alcohol solution of echinochrome.

Sp. 10.—What was left untouched by the chloroform went into ether, giving this spectrum.

Sp. 11.—An absolute alcohol solution was evaporated down, residue dissolved in chloroform ; this was evaporated, and the residue dissolved in ether gave this spectrum, which differs from Sp. 10.

Sp. 12.—Bisulphide of carbon solution of the residue left from the evaporation of a chloroform solution.

Sp. 13.—Dried clot in ether.

Sp. 14.—Dried clot in chloroform.

Sp. 15.—Dried clot in bisulphide of carbon.

Sp. 16.—Dried clot in benzene.

Sp. 17.—Glycerin extract of fresh echinochrome.

Sp. 18.—The same with stannous chloride.

A comparison of these spectra shows how remarkably unstable echinochrome is ; it is on this instability that its usefulness as a respiratory substance depends.

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[1] This band is rather too dark.

B C   D   E b   F   G

Sp. 1.		Oxychloro- cruorin.
2.		The same + NH₄ HS
3.		N⁰ 2 + Na HO
4.		Aq. sol + Na HO then NH₄ HS
5.		Aqueous solution of Oxychlorocruo⁰
6.		The same + N H₄ H
7.		Blood of Serpula living animal.
8.		Blood of another Serpula.
9.		D⁰ from a dilated part of blood ves
10.		D⁰ from same pa a third specime
11.		D⁰ from same pa a fourth specim
12.		Aqueous solutio of blood of Serp
13.		D⁰ other Specimens
14.		D⁰ + Na HO, the N H₄ H S.
15.		Gills of Serpul
16.		Operculum of Serpula.

The Cephalic Appendages of the Gymnosomatous Pteropoda, and especially of Clione.

By

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Brussels.

With Plate XXXV.

Eschricht formerly made known, on the three pairs of cephaloconi ("Kopfkegel") of Clione borealis, some structures which he described and represented as real suckers; at the same time he drew attention to the analogy of their situation with that of the suckers of the Cephalopoda.[1]

This fact was of great importance, since it accorded with the presence of suckers on the two buccal appendages of another Gymnosomate, viz. Pneumodermon, and gave great support to the opinion expressed by R. Leuckart, that the six conical appendages of the head of Clione correspond with the arms of the Cephalopoda.[2]

From that time the assertion that Clione possesses acetabuliferous appendages has been admitted everywhere, and it is found reproduced in the most valuable and most recent works.

However, the excessive smallness of the structures which Eschricht had described as suckers (he attributes to them 0·005‴ of diameter) permitted one to call in question their assimilation with the suckers of Pneumodermon and of the

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[1] Eschricht, 'Anatomische Untersuchungen über Clione borealis,' p. 9.

[2] R. Leuckart, 'Ueber die Morphologie und die Verwandschaftsverhältnisse der Wirbellose Thiere.'

Cephalopoda, the more so as Hobböll, who has observed Clione living, and has often seen their cephaloconi expanded, has never remarked that these animals fixed themselves by these appendages,[1] while several naturalists have seen Pneumodermon in life, and have noticed that it frequently fixes itself with the aid of its acetabuliferous appendages.

Therefore, during the sojourn which I made in the winter of 1884-85 at the zoological laboratory of University College (London), Professor Ray Lankester proposed to me to study the structure of the cephaloconi of Clione.

Having set to work, I immediately saw that in a large number of well-known and even very recent works great confusion prevailed on the cephalic appendages of Clione. The reason of this is specially to be found in the imperfection of the original figures, which are generally obscure on the subject; and those which are most often reproduced are just the most defective,[2] for they give a very bad idea of the cephalic appendages of Clione, or are even absolutely incomprehensible on this point.

Those of Eschricht are small, and also difficult to understand, so that the reproductions which have been made of them are inexact, and, as Keferstein has remarked, "all those processes which have been named tentacles by Eschricht want a new description." [3]

When I had comparatively examined other Gymnosomata, I remarked that there also a confusion quite as great existed on the cephalic appendages, the more so since the original authors do not agree on this question.

In order to dispel the confusion which exists on this subject, I have thought useful to represent on a rather large scale, and under different aspects, the cephalic part of some

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[1] Eschricht, loc. cit., p. 9.

[2] For example, the figure of Clione given by Rang (Rang et Souleyet, 'Histoire naturelle des Mollusques Ptéropodes,' pl. vii, fig. 9), reproduced by Keferstein (Bronn's 'Thierreich'), by Claus ('Elementary Text-Book of Zoology '), &c.

[3] Keferstein, Bronn's 'Thierreich,' Abth. iii, p. 613.

Gymnosomata. I thus have been induced to extend to the cephalic appendages of the gymnosomatous Pteropoda a work specially undertaken to make known the structure of the cephaloconi of Clione.

This paper is divided into three parts, corresponding to the different genera which I have studied: Clione, Clionopsis, and Pneumodermon.

I. Clione.[1]

Clione (Pl. XXXV, figs. 1—4) possesses two kinds of cephalic appendages:

    1. Tentacles properly so called.

    2. Cephaloconi or buccal cones.

I shall examine successively these two orders of appendages.

A. Tentacles properly so called.

Clione possesses two pairs of them—an anterior or labial pair, and a posterior or nuchal pair.

Anterior Pair.—It is situated on a hood, whose two halves, right and left, may fall back laterally, or be joined together again on the antero-posterior mesial line, and hide the buccal opening and the three pairs of buccal cones. These tentacles are long and retractile. They are not absolutely anterior, as in the figure of Eschricht, which represents them as equally distant from the dorsal and ventral faces;[2] they are situated nearer the dorsal than to the ventral face, as shown in Plate XXXV, fig. 2.

Very powerful longitudinal muscles occupy the whole of the interior part of these appendages; externally, we find a thin layer of annular muscular fibres. The epithelium is like that of the other parts of the body; their cells are cylindrical and provided with a large nucleus.

Sections which pass towards the free extremity of these tentacles show a rather large number of nervous cells, but I have

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[1] The species studied is Cl. limacina, Phipps, = Cl. borealis, Pallas.

[2] Eschricht, loc. cit., Taf. 2, fig. 10.

not seen any special nervous terminations, such as we shall find on the buccal cones.

Posterior Pair.—It is situated on the dorsal face of the neck. These tentacles are much shorter than those of the first pair. Upon the animals preserved in spirit they are always retracted, so that they are difficult to see, their presence being then disclosed only by two slight recesses, from which their extremities are sometimes seen emerging under the form of a white point.

According to Eschricht, this second pair is oculiferous ;[1] but this fact has been called in question by several naturalists, and categorically denied by von Ihering.[2]

The histological examination which I have made of the nuchal tentacles of Clione, Clionopsis, and Pneumodermon, allows me to assert that these appendages do present eyes at their free extremity.

Unfortunately the specimens which have served me in my researches had not been specially prepared for the study of the nervous system and of nervous terminations as delicate as the retinal ones. It follows that I can neither draw nor describe in a complete manner the structure of the eyes of the Gymnosomata. That is a point which I intend to take up as soon as possible.

Nevertheless, the profound dissimilitude which I have observed between the labial and nuchal tentacles, and the characters of the structure of the latter in the three species which I have studied, show that the nuchal tentacles are quite different from those of the first pair, and the presence of a refracting body shows that the sense of which they are the seat is that of sight.

At the free extremity of these tentacles, the epithelium becomes much thinner, so as to make a pellucida or cornea. Under this membrane we find a spherical lens, of which the structure is similar to that of the Pulmonata. As I have

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[1] Eschricht, loc. cit., taf. 3, fig. 29.

[2] Von Ihering, ‘Vergleichende Anatomie der Nervensystem und Phylogenie der Mollusken,’ p. 240.

already said, the retinal part is not perfect in any section, but its position and general form recall to mind those of the retina of the Gastropoda. In the specimens of Clione the pigment had disappeared, but in those of Pneumodermon it was preserved. Finally, towards the base of the tentacle the nerve traverses an optic ganglion.

B. Buccal Cones.

They number three pairs, symmetrically situated on the two sides of the "lips." The two dorsal cones are the longest, the two ventral cones the shortest (fig. 3).

These cones are very extensible, and in the state of expansion they are much longer than they are generally represented. They are absolutely conical, as my figures show them, which I made from specimens[1] of the "Challenger," preserved with the extended cones and all the appearances of the living animal.

These cones are brightly coloured during life. They are inserted on the two sides of the "lips" as shown in fig. 4.

They are hollow in their lower half, and their cavity is continuous with that of the head, which includes the buccal mass and the penis. Examining these cones with a magnifying glass, one sees that they are covered with innumerable very small tubercles, which have been described by Eschricht as so many groups of suckers.

Structure of the Buccal Cones.

On any section of a buccal cone of Clione we may easily distinguish three different regions :

1. A middle, muscular region formed of two parts: *a.* an exterior layer with annular fibres (fig. 11, *a*); *b.* an interior layer with longitudinal fibres (fig. 11, *a'*).

2. An internal region formed of glandular cells (fig. 11, *b*).

3. An external region, or epithelial clothing (fig. 11, *c*).

[1] Mr. John Murray, F.R.S.E., Director of the Challenger publications, kindly supplied Professor Lankester with these specimens as soon as he heard that Clione was undergoing investigation in the laboratory of University College.

I shall examine these different parts successively.

1. M u s c l e s   o f   t h e   B u c c a l   C o n e s.—The muscular cells
are unstriped, elongated, and contain a nucleus of a prismatic
form (fig. 13, b'). The muscular external layer of annular fibres
is much less developed than the internal layer. This latter is
very powerful, which explains the great extensibility of the
cones. The longitudinal fibres are united in distinct groups
(fig. 13, b).

2. G l a n d u l a r   I n t e r n a l   C e l l s.—A transversal section of
one of the cones shows that their interior is filled with cells
united in groups (fig. 11, b). In the lower half of the cone
the centre of the sections is empty, and the cells are only
found against the longitudinal muscular layer.

A longitudinal section will make this disposition better
understood (fig. 12). The cells in question are united in
elongated groups, having the form of follicles. Each cell
possesses a proper prolongation, which is continued to the
epithelial covering of the cone. Each of these groups possesses
a basement membrane of connective nature, but the different
groups are pressed one against the other without one being
able to see between them any free connective tissue, under
the form of cells or fibres. The spaces which are seen in
several places, on the figure 10 of the plate, proceed from dis-
placements which occur during the preparation of the sections.
Among the groups which have been displaced I have not seen
any traces of connective tissue.

These groups of secreting cells do not constitute a gland,
for nowhere on any section, longitudinal (fig. 16) or transversal
(fig. 17), can a lumen be seen, nor efferent duct. Each cell
is an independent unicellular gland. The contents of these
cells is a slightly granular substance. The nucleus is large
and spherical; it gives indications of its reticulated structure,
but not clearly enough to make drawings of them showing this
structure.

The cells situated at the interior extremity of the groups
have excessively long prolongations (fig. 16, d); the cells
situated near the muscular layer have, on the contrary, much

shorter ones. When these prolongations arrive at the longitu-
dinal muscular layer their contents change their appearance
and present themselves under the form of a fibroid secretion
(fig. 18, c) which absorbs much hæmatoxylin. These fibroid
prolongations pass between the groups of longitudinal muscular
fibres (d, fig. 13), traverse the layer of circular muscles, and
pass into the reticulum of subepithelial connective tissue;
afterwards each prolongation penetrates into an epithelial cell,
which it traverses by passing between the membrane and the
cellular contents.

3. Epithelial Investment.—This is the most character-
istic part of the buccal cones of Clione. The epithelial cells
are united in a variable number, so as to form an infinity of
small circular groups on the surface of the cones. It is these
small groups which give to the cones their rugose or wrinkled
aspect.

A transversal or longitudinal section shows that these
groups, pressed one against the other, cover the whole surface
of the cone, and that each group is formed of a little elevation
upon which the epithelial cells are found.

Examining one of these groups with an ordinary magnifying
power (Verick, obj. 6) we see that the space between the
annular muscular fibres and the epithelial cells is occupied by
a reticulum of connective tissue which unites the two above-
named elements.

The epithelial cells (fig. 13, f) are elongated, nearly cylindrical,
but wider towards their lower part. They are separated from
one another at their higher part, and end at their free extre-
mity in a button-like enlargement.

The cellular contents (h) have nearly the form of the cell.
At the lower part the contents have the form of a club, the
big end of which would be turned towards the bottom; at
the higher part the contents fill exactly the terminal enlarge-
ment.

This cellular substance is finely granular, but does not com-
prise any nucleus.

The cellular membrane is rather thick and presents, in its

thickness, longitudinal striæ which are strongly coloured by the hæmatoxylin.

Between the cellular substance and the membrane we find the fibroid secretion (d) of the glandular cells which occupy the interior of the cone.

Each of the epithelial cells so constituted has been taken by Eschricht for a sucker.

Under each epithelial group, at the surface of the annular muscular layer, or even between the fibres of the latter, is found a large sensorial cell (i), which sends a prolongation (k) across the subepithelial connective tissue. This prolongation continues between the epithelial cells and terminates freely at the surface of the cone.

These sensorial cells possess a large refracting nucleus (j), with a strongly colourable nucleolus.

The prolongation, rather narrow in the connective tissue, enlarges a little between the epithelial cells and constitutes a rod in the form of an elongated cone (l), with the base turned towards the surface. The part of the prolongation, contained in the connective tissue, presents in some series of sections strongly coloured, a very special aspect; it appears to be reticulated (fig. 14, a). The conical part, situated between the epithelial cells, presents numerous longitudinal striæ. The rod is terminated by a kind of small horizontal rather thin disc (fig. 13, m), which is more strongly coloured than the subjacent part. This disc does not bear cilia such as we see upon the extremity of some sensorial cells. I do not think that in the specimens I have studied, the sensorial cells have lost their ciliary covering, for the other parts of the body which bear ciliæ have their ciliary investment intact.

At the prolongation of the sensorial cell, towards the base of the epithelial cells, we find a spherical or ovoid refracting body (n) joined to it, whose membrane seems rather thick, and in the interior of which we find a corpuscle deeply coloured by the hæmatoxylin.

The complicated structure of these epithelial groups is very clearly shown by a series of transversal sections of these groups,

that is to say, by sections made tangentially on the surface of the cone. Such sections are difficult to obtain exactly, but when they are in the direction wished for, they are very instructive.

I represent four of these sections, which I shall describe successively, passing from the base to the free extremity.

1. Section passing above the annular muscular layer (fig. 19). We see the sensorial cell (*a*) in the middle of the reticulated connective tissue (*b*), in which we find also fibroid prolongations (*c*) of the internal glandular cells.

2. Section passing through the base of the epithelial group (fig. 20). Here we see the prolongation (*a*) of the sensorial cell, the continuation of the fibroid prolongations (*c*), and the surrounding connective tissue (*b*).

3. Section passing through the extreme base of the epithelial cells (fig. 21). We find the rod (*a*) at the centre, with the refracting body (*b*), which is joined to it. All around are the bases of the central epethelial cells (*c*), in the interior of which we see the fibroid secretion (*d*) of the internal glandular cells. At the external part one sees connective tissue (*e*).

4. Section passing through the epithelial cells (fig. 22). The external cells are already separated from their neighbours. We see in the middle the section of the rod (*a*), of which we distinguish the striated structure. In the membrane (*b*) of the epithelial cells we find some coloured points (*c*), indicating the sections of the longitudinal striæ in this membrane, which I have already described.

Summary.—What Eschricht has taken for suckers are epithelial cells terminated by a button-like enlargement. It is noticeable that there is no nucleus to be observed in these cells. Besides, they are penetrated by the secretion of the glandular cells which occupy the interior part of the cone. The latter are so numerous (apparently as numerous as the cells of the epithelial groups) that they doubtless fulfil important functions. I think that their secretion is spread outside of the cone across the button-like extremity of the epithelial cells, for on some specimens of which the epithelium

has remained quite intact this secretion had spread outwardly, had become coagulated under the influence of the spirit, and had formed a stratified deposit, which absorbs much colouring matter on the surface of the cone.

Each group of epithelial cells is provided at its centre with a sensorial cell with a rod-like prolongation. This fact determines the buccal cones of Clione as organs of special sensibility. A refracting body is invariably found towards the central part of the epithelial groups at the base of the cells; it is also probably, on account of its situation in contact with the rod, an integral part of the sensorial apparatus.

With respect to the special nature of these organs, I may make a remark with reference to the sense of smell in aquatic animals. Is smelling possible in water, such as exists among the superior Vertebrata? I do not think so. There must be a special sense of which Mammalia cannot be conscious; and a truly aquatic animal cannot have an idea of the smelling of aërial animals. This sense must be a peculiar one, intermediate to that of smell and that of taste. The buccal cones of Clione are probably the seat of such a sense.

II.—Clionopsis.[1]

Clionopsis only possesses one kind of cephalic appendages—tentacles properly so called.

As with Clione, there are two pairs—a labial pair and a nuchal pair (fig. 5).

The labial tentacles are less elongated than with Clione. As in the latter they are inserted more dorsally than ventrally. Their structure is that of the corresponding appendages of Clione.

The nuchal pair has already been described by Troschel.[2] Gegenbaur says that it is absent.[3] Upon the specimen which

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[1] The species studied is Clionopsis Krohni, Troschel, = Clio. mediterranea, Gegenbaur.

[2] Troschel, " Beiträge zur Kenntniss der Pteropoden," 'Arch. für Naturg.,' 1854, p. 229.

[3] Gegenbaur, 'Untersuchungen über Pteropoden und Heteropoden,' p. 70, Taf. iv, fig. 14.

I have studied the nuchal tentacles were extended and very easily visible. Their structure is that of the nuchal tentacles of Clione. The eyes are not situated near the tentacles, as Troschel says,[1] but on their top.

Clionopsis does not possess any other cephalic appendages. The lips open into a narrow buccal cavity, and do not form a hood which can fall back, as with Clione.

III. Pneumodermon.[2]

Pneumodermon is provided with two kinds of cephalic appendages:

1. Tentacles properly so called, corresponding to those of Clione and Clionopsis.

2. Two acetabuliferous buccal appendages, characteristic of the genus (figs. 8, 9).

A. Tentacles properly so called.

Like Clione and Clionopsis, Pneumodermon possesses two pairs of tentacles, a labial pair and a nuchal pair.

As with the two preceding genera, the labial tentacles are found situated on the two sides of the mouth, rather dorsally.[3]

The nuchal pair has already been described by Souleyet.[4] Gegenbaur asserts that the first pair is the only one which exists.[5] The second pair is, indeed, very difficult to see, being retracted in the preserved specimens, the recesses which result from it being imperceptible; however, this place is less coloured than the adjacent parts. By making some transversal sections in the anterior part of Pneumodermon, I

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[1] Troschel, loc. cit., pl. x, fig. 9, *o*.

The species which I studied are : Pneumodermon mediterraneum, van Ben., and P. Peronii, Lam.

[3] Figure 7 may be applied to Pneumodermon as well as to Clionopsis. Besides Souleyet ('Zoologie du voyage de la Bonite,' pl. xv, fig. 15) has already very well represented this aspect of Pneumodermon.

[4] Loc. cit., vol. ii, p. 256, and pl. xv, fig. 14.

[5] Gegenbaur, loc. cit., p. 24.

have been able to assure myself with certitude that the second pair of tentacles positively exist in this genus, as with Clione and Clionopsis. But I have not remarked that they had the bifid form indicated by Souleyet.[1] These tentacles bear at their free extremity an eye, which has the same structure as those of the two preceding genera.

B. Acetabuliferous Buccal Appendages.

These appendages are inserted on the internal wall of the buccal cavity, on the ventral side. Two figures of Souleyet show this disposition perfectly (loc. cit., pl. 15, figs. 17 and 30); fig. 17 has been badly understood by Fischer, who gives the two groups of suckers for the jaws.[2]

The acetabuliferous appendages of Pneumodermon have the form of a flattened cylinder, upon which are inserted the peduncles of the suckers. These vary in number according to the species. Pneumodermon Peronii possesses a large number of them, about thirty on each appendage; P. violaceum, ten to fourteen on each appendage, and P. mediterraneum five or six. However, in this last species I have sometimes found seven suckers, but then one or two were very small.

In the state of inactivity the suckers have the form of a very flat porringer. It is in P. mediterraneum that I saw the largest; they were one line in diameter.

Structure of the Acetabuliferous Buccal Appendages.

It does not at all resemble that of the buccal cones of Clione. The whole of its mass is formed by longitudinal muscular fibres. Externally we find a uniform epithelium; that is to say, it is not provided with sensorial cells like the epithelium of the cones of Clione. The buccal appendages of Pneumodermon are not, then, sensorial organs, as some

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[1] Loc. cit., vol. ii, p. 256.
[2] Fischer, 'Manuel de Conchyliologie,' fig. 42, p. 44.

authors say.[1] Moreover, there is nothing in these appendages which recalls to mind the internal glandular mass of the cones of Clione.

Structure of the Suckers.

The only histological knowledge of these organs which we formerly possessed proceeds from Gegenbaur's researches, who attributes a very simple structure to them.[2] But this knowledge was not very extensive.

During the month of January, 1885, I studied the structure of these suckers by means of a series of transversal sections. I had scarcely finished this study when I received the thesis of Niemiec, " Recherches morphologiques sur les ventouses dans le règne animal."[3] In this work the structure of the suckers of Pneumodermon is carefully described and represented. My own observations agreeing almost entirely with those of the Swiss zoologist, I believe it useless to explain them at length. Hence I refer to the memoir of Niemiec, limiting myself to the principal points relative to the structure of the sucker and to indicating a few points of detail in which I do not entirely agree with the Swiss zoologist.

The body of the sucker is formed of a layer of large, prismatic, muscular cells (figs. 24 and 25), the contents of which are formed of different separated fasciculi of fibres (a). These fasciculi have the form of very flat, three-sided prisms, of which the most acute angle is turned towards the centre of the cell. In this place we find the nucleus (b), which has a shape analogous to that presented by the nucleus of the muscular cells of the cones of Clione. All around, towards the upper side of the disc formed by this layer of prismatic cells, we find a sphincter formed of not very numerous annular muscular fibres. The upper face of the muscular disc is covered with a pavimentous epithelium (fig. 23, c) with excessively flattened cells, whose nucleus is consequently excessively flat.

[1] Claus, ' Handbuch der Zoologie,' French translation, p. 1056.
[2] Gegenbaur, loc. cit., p. 77.
[3] ' Recueil zoologique suisse,' t. ii, 1885.

Between this epithelium and the prismatic muscular cells we see absolutely no connective tissue. On the upper side of the disc, outside the sphincter, the epithelial lining thickens very much, which is explained by the fact that it is by this part that the adherence of the sucker to foreign bodies is produced.

I have not remarked the constant presence of the cuticular pads ("bourrelets") which Niemiec described.[1] Perhaps these parts are specially visible in suckers of large size.

Beneath the epithelial thickening of which I speak, we find, all around the disc, glandular cells in the form of a flask with a very narrow neck. The efferent duct of these cells traverses the epithelial thickening and passes out at the upper face of the exterior ring of the sucker, that is to say, on the point where the adherence is produced. The secretion of these cells probably makes this adherence more perfect.

On the lower face of the sucker the epithelium is less flattened than on the upper face, and it is united to the prismatic muscular cells by connective tissue.

The peduncle is covered with an epithelium continuous with that of the upper face of the sucker, and quite analogous to it. The peduncle itself is formed by the continuation of the longitudinal muscular fibres of the buccal appendage. These fibres turn to the lower part of the acetabular disc.

According to Niemiec, some fibres of the peduncle go to the side of the sucker. Such fibres I have not observed. In my opinion the longitudinal muscular fibres of the peduncle (fig. 23, *a*) only go to the central part of the disc, and are inserted on it, between two prismatic muscular cells, by their extremity, which ends in a point (*b*). A retracting muscle inserted on the side of the sucker would be far from having a useful effect. Since it is at this point that the adherence is produced, the action of such muscles could only tend to combat it, while the muscles inserted on the centre of the sucker, by removing this point from the body to which the circumference

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[1] Loc. cit., pl. iii, fig. 2, *c*.

of the disc adheres, augment the vacuum under this disc, and consequently the adherence.

Except this point of detail, I quite agree with Niemiec on the physiological mechanism of the suckers of Pneumoderm on. I am specially persuaded that the prismatic muscular cells fulfil an important part during the first moments of fixation. These cells are especially worthy of the attention of those who are interested in the comparative study of different forms of muscular tissue.

Cirrifer.

In 1879, G. Pfeffer described by this name a gymnosomatous Pteropod, which much resembles Pneumodermon, whose caudal and lateral gills it possesses.[1]

This Pteropod bears two buccal appendages like Pneumoderma, but instead of being provided with suckers these two appendages are terminated by two small branches bent round in the form of a sickle. According to the drawing of Pfeffer, these appendages differ further from those of Pneumodermon, in that they are not inserted separately on the buccal wall, but reunite in a common stem before arriving at that wall.

Pfeffer describes two superior or labial tentacles ; it is very probable that the nuchal tentacles, very small and retracted, have escaped him, as those of Pneumodermon have escaped Gegenbaur.

Summary.

The different authors agree but little on the cephalic appendages of the gymnosomatous Pteropoda, and many of them consider all these appendages as tentacles. In short their homologies are very obscure.[2]

We have stated that in the Gymnosomata there exists in a constant manner two pairs of tentacles properly so called. I

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[1] 'Monatsberichte der Akad. der Wissensch.,' Berlin, 1879, p. 249, fig. 20.

[2] "The connections between these conformations and the tentacles of the Gastropoda are not yet very clear," Gegenbaur, 'Grundzüge der Vergleichenden Anatomie,' French translation, p. 481.

do not think it will be rash to identify these appendages with the two pairs which the Gastropoda Euthyneura (Opistho-branchia and Pulmonata) possess, and which occupy the same position among these animals as with the Gymnosomata.

In the Thecosomata we find a pair of rudimentary ten-tacles, for example, in Hyalaea, Cleodora, and Creseis,[1] Cuvieria and Spirialis,[2] Tiedemannia[3] and Cymbulia.[4]

With several of these animals the tentacles present rudi-mentary eyes, Creseis for example.[5]

If they do not present eyes in the adult state they possess them in some stage of the development, as in Tiedemannia and Spirialis.[6]

This pair of tentacles is, in my opinion, equivalent to the oculiferous nuchal pair of the Gymnosomata. As to the anterior pair, its disappearance is explained by the displace-ment of the swimming lobes, which encircle the head and between which the mouth opens. The development of the fins in this position has caused the disappearance of the anterior tentacles.

Besides the two pairs of tentacles properly so called, we have seen that most of the Gymnsomata possess buccal appendages; such are Clione, Pneumodermon, Cirrifer.

Without prejudging anything as to the morphological value of these appendages, I believe that they have the same origin, however varied their aspect may be in the three genera above named.

Apparently it seems that with Clione they are inserted around the mouth, while with Pneumodermon and Cirrifer they are inserted on the internal wall of the buccal cavity. But it should be noted that in Clione there exists a hood which can fall back. This hood covers the buccal cones, and its opening corresponds to the buccal opening of Clionopsis,

[1] Gegenbaur, 'Untersuchungen über Pteropoden,' p. 8.

[2] Souleyet, loc. cit., vol. ii, pp. 199 and 209, pl. xii, fig. 32, and pl. xi, fig. 15.

[3] Gegenbaur, loc. cit., p. 60.

[4] Gegenbaur, loc. cit., p. 45.

[5] Gegenbaur, loc. cit., p. 8, Taf. ii, fig. 1.

[6] Kröhn, 'Beiträge zur Entwickelungsgeschichte der Pteropoden,' p. 21.

Pneumodermon, and Cirrifer (compare figs. 2, 4, and 7) The "lips" situated between the cones of Clione are not then equivalent to the lips of the other Gymnosomata, in which there is no developed hood like that of Clione. They would be a differentiation of the internal wall of the buccal cavity, which is produced behind the buccal appendages.

In this manner we see that with Clione, as with Pneumodermon and Cirrifer, the buccal appendages are inserted on the internal wall of the buccal cavity.

At the same time it must be remembered that this front portion of the buccal cavity may be regarded as not part of the true oral cavity, but as only an "introvert" like that of proboscidiferous Gastropods.

EXPLANATION OF PLATE XXXV.

Illustrating Dr. Paul Pelseneer's Paper on "The Cephalic Appendages of the Gymnosomatous Pteropoda, and especially of Clione."

FIG. 1.—Head of Clione. Dorsal aspect. *a*. Labial tentacles. *b*. Nuchal tentacles. *c*. Buccal cones. *d*. Fins. *e*. "Lips." *f*. Edge of the hood.

FIG. 2.—Head of Clione. Oral view, the hood being nearly closed. *a*. Labial tentacles. *c*. Buccal cones. *f*. Edges of the hood.

FIG. 3.—Head of Clione. Lateral view. *a*. Labial tentacle. *b*. Nuchal tentacle. *c*. Buccal cones. *d*. Fin. *e*. Foot. *f*. Edge of the hood. *g*. Orifice of the penis.

FIG. 4.—Head of Clione. Oral view, the hood being open. *a*. Labial tentacles. *c*. Buccal cones. *d*. "Lips." *f*. Edge of the hood.

FIG. 5.—Head of Clionopsis. Dorsal aspect.

FIG. 6.—Head of Clionopsis. Lateral view. *a*. Labial tentacles. *b*. Nuchal tentacles. *c*. Foot. *d*. Fins. *e*. Orifice of the penis.

FIG. 7.—Head of Clionopsis. Oral view. *a*. Labial tentacles. *b*. Mouth. *c*. Lips.

Fig. 8.—Head of Pneumodermon.   Dorsal aspect.

Fig. 9.—Head of Pneumodermon.   Lateral view.   *a*. Labial tentacles.
b. Nuchal tentacles. *c*. Acetabuliferous buccal appendages. *d*. Fins. *e*. Lips.
f. Foot. *g*. Orifice of the penis.

Fig. 10.—Head of Creseis. Dorsal aspect. (After Gegenbaur.) *a*. Mouth.
b. Tentacles with eyes. *c*. Foot. *d*. Fins. *e*. Mantle.

Figs. 11—22.—Clione.

    Fig. 11. Transverse section of a buccal cone. *a*. Annular muscular
    layer. *a'*. Longitudinal muscular layer. *b*. Internal glandular cells.
    c. Epithelial covering.

    Fig. 12. Longitudinal section of a buccal cone. Letters as in Fig. 11.

    Fig. 13. An epithelial group of the section Fig. 11, more magnified.
    a. Annular muscular layer. *b*. Longitudinal muscular layer. *b'*. Nu-
    cleus. *c*. Internal glandular cells. *d*. Their fibroid secretion. *e*. Re-
    ticulated connective tissue. *f*. Epithelial cells. *g*. Its button-like
    termination. *h*. Cellular contents. *i*. Sensorial cell. *j*. Its nucleus.
    k. Its rod-like prolongation. *l*. The striated conic part of the rod.
    m. The terminal disc of the rod. *n*. Refracting body.

    Fig. 14.—An epithelial group, showing the reticulated aspect of the rod
    of the sensorial cell. *a*. Prolongation of the sensorial cell. *b*. Striated
    part of the rod. *c*. Refracting body. *d*. Epithelial cells. *e*. Fibroid
    secretion of the internal glandular cells.

    Fig. 15.—An epithelial cell, showing the striæ of the membrane.
    a. Striæ of the cellular membrane. *b*. Cellular contents. *c*. Fibroid
    secretion.

    Fig. 16.—Longitudinal section of a group of glandular cells of the in-
    terior of a buccal cone. *a*. Internal glandular cells. *b*. Its nucleus.
    c. Basement membrane. *d*. Prolongation of the glandular cell.

    Fig. 17.—Transversal section of several groups of internal glandular
    cells. *a*. Glandular cells. *b*. Nucleus. *c*. Basement membrane.

    Fig. 18.—A glandular cell adjacent to the muscular part of the cone,
    showing the passage of the cellular substance to the fibroid secretion.
    a. Nucleus. *b*. Cellular contents. *c*. Fibroid secretion.

    Fig. 19.—Transverse section of an epithelial group, passing a little
    above the annular muscular layer. *a*. Sensorial cell and its nucleus
    b. Reticulated connective tissue. *c*. Fibroid secretion of the internal
    glandular cells.

    Fig. 20. Ditto, through the base of the group. *a*. Prolongation of the
    sensorial cell. *b*. Reticulated connective tissue. *c*. Fibroid secretion
    of the internal glandular cells.

    Fig. 21. Ditto, through the extreme base of the epithelial cells. *a*. Pro-
    longation of the sensorial cell. *b*. Refracting body. *c*. Base of the
    central epithelial cells. *d*. Fibroid secretion. *e*. Connective tissue.
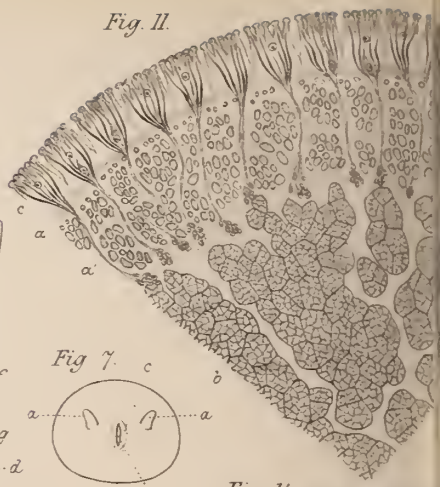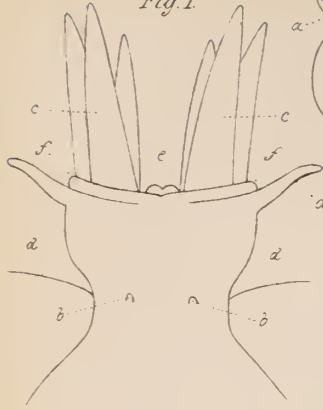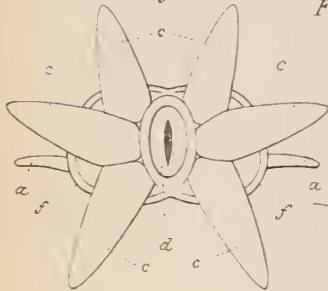
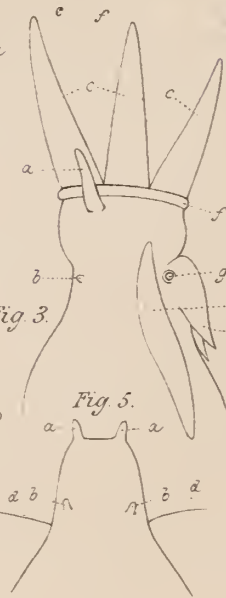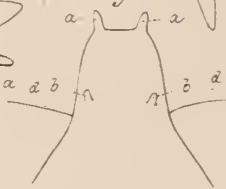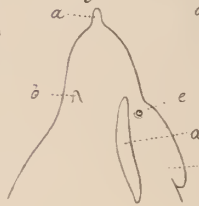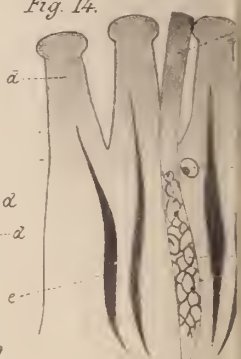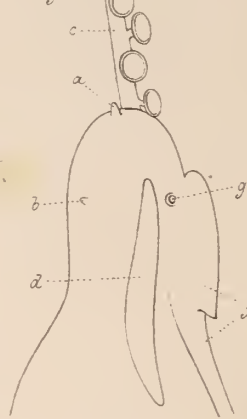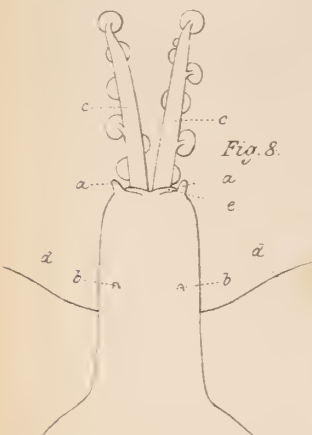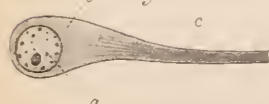Fig. 1. Fig. 2. Fig. 11. Fig. 4 Fig. 3. Fig. 7 Fig. 5. Fig. 6. Fig. 14. Fig. 8. Fig. 9. Fig. 10. Fig. 18. Fig. 21. Fig. 20. Fig. 22. Fig. 19.
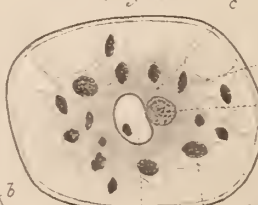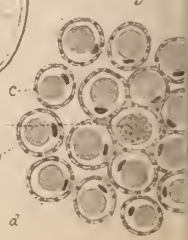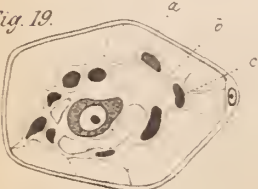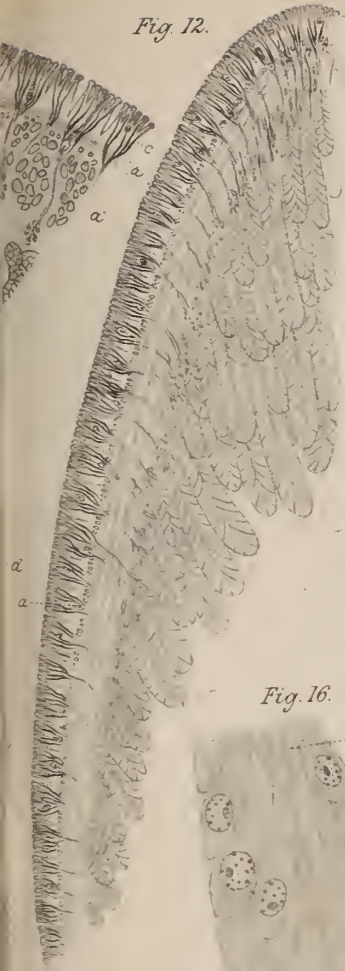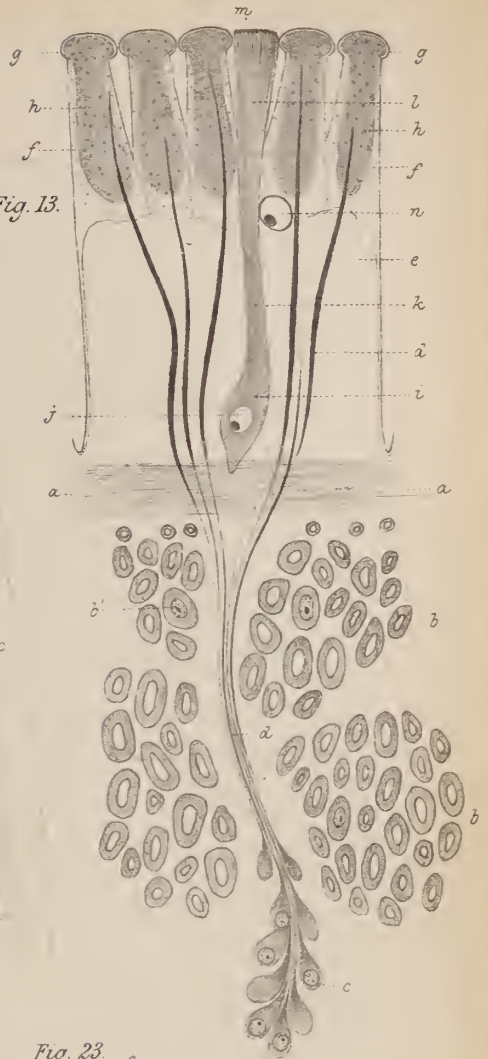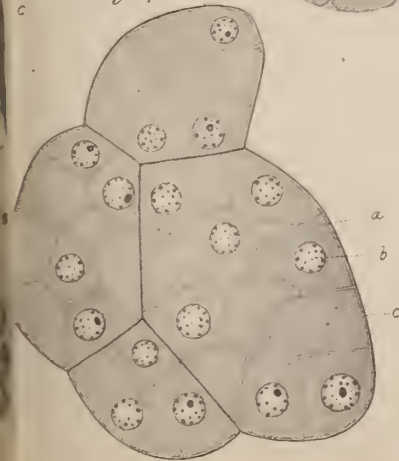
Paul Pelseneer del

Fig. 12.

Fig. 13.

Fig. 15.

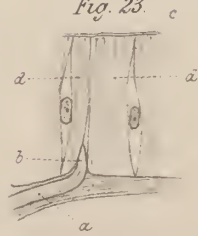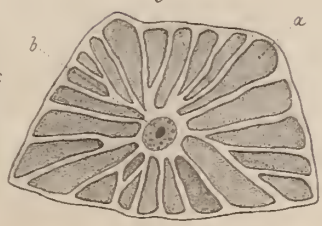Fig. 16.
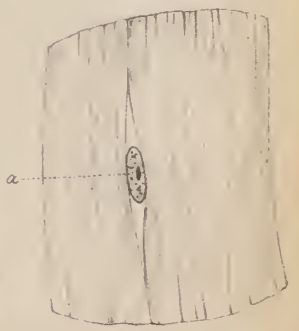
Fig. 17.

Fig. 23.

Fig. 24.

Fig. 25.

Fig. 22. Ditto, through the body of the epithelial cells.   *a.* Rod or prolongation of the sensorial cell.   *b.* Membrane of the epithelial cells.   *c.* Striæ of this membrane.   *d.* Fibroid secretion.

FIGS. 23—25.—Pneumodermon.

Fig. 23. Part of a transverse section of a sucker, showing how the longitudinal muscles of the peduncle are inserted on the muscular disc.   *a.* Longitudinal muscular fibre of the peduncle.   *b.* Its pointed extremity.   *c.* Epithelium of the upper face of the sucker.   *d.* Prismatic cells of the muscular disc.

Fig. 24. Transverse section of a prismatic muscular cell.   *a.* Prism of muscular fibres.   *b.* Nucleus.

Fig. 25. Longitudinal section of a prismatic muscular cell.   *a.* Nucleus.
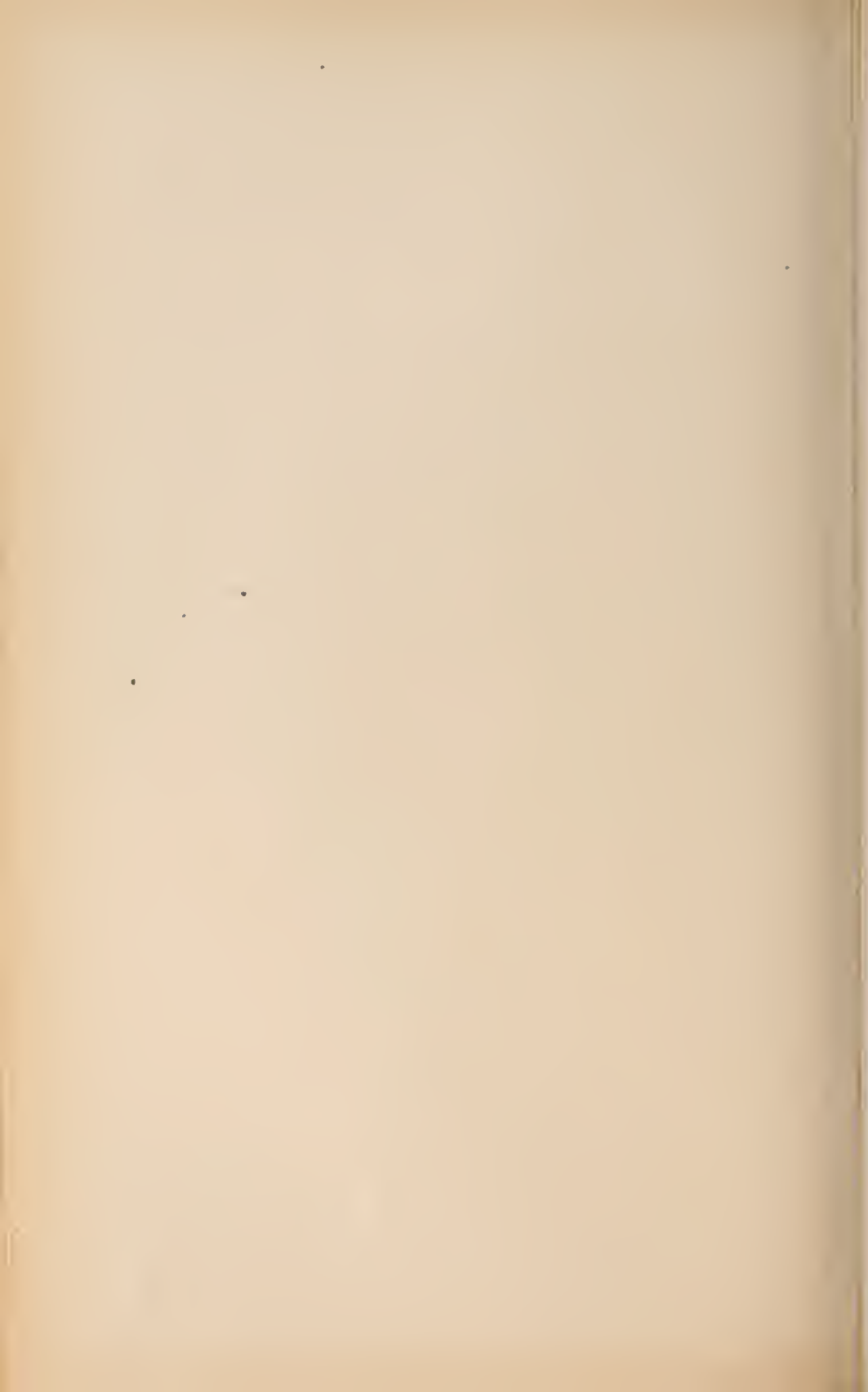
Evidence in favour of the View that the Coxal Gland of Limulus and of other Arachnida is a Modified Nephridium.

By

G. L. Gulland, M.A., B.Sc.

With Plate XXXVI.

THE following observations were made by the author whilst acting as assistant to Professor Lankester in connexion with a research on the comparative histology of the vascular system and connective tissues of Arthropoda and Mollusca, in aid of which a grant was made by the Government Grant Committee of the Royal Society. The specimens of Limulus, of various sizes, from a quarter of an inch diameter upwards, were very kindly procured for Professor Lankester by Professor H. Newell Martin, of Baltimore.

In a transverse section of a young Limulus of the size represented by fig. 3, *a* at the level of the 4th or 5th appendage (such as is represented diagrammatically in fig. 1), the observer's eye is at once caught by three or four large spaces lined by a peculiar epithelium, in the connective tissue immediately external to the entosternite and on the same level with it. This is the representative of the coxal gland. As we had a complete series of transverse sections of the prosoma, we proceeded to reconstruct the gland, by making sketches of the spaces at frequent intervals, colouring the drawings so as to ensure the recognition of each space through all its changes, and from this series of sketches by drawing to a certain scale we compounded a diagram which is reproduced in fig. 2.

It must be borne in mind that the tubes though necessarily drawn on one plane in order to show the relation of the whole, are really clustered together, so that the true appearance of the gland, if dissected out, would be more like that shown in fig. 3, *cogl*. From this diagram it will be seen that the gland consists of a tube which, opening posteriorly at a point to be referred to presently, passes forward towards the anterior end of the body as a simple tube, is bent upon itself, passes again towards the posterior end of the body, and on its way gives rise to several secondary tubes, which in their turn have outgrowths. The process of budding and division is best seen in the tubes at the right hand of the diagram where there are several commencing outgrowths, and one tube is being divided into two by numerous septa which pass across its lumen.

These tubes are completely closed and are lined by an epithelium, to be described afterwards, except at one point where the wall is deficient and the lumen of the tube is continuous with the connective-tissue spaces which everywhere surround the gland. (The details of this are shown in figs. 4 to 9, and will be described later on.)

The relations of the gland to surrounding structures approximate pretty closely to those of the gland in the adult. There is, of course, no trace of the lobes which correspond to the coxæ of the second, third, fourth, and fifth limbs, but the anterior end of the gland reaches as far forward as a point corresponding to the posterior edge of the coxa of the second limb, while the cæcal posterior end reaches to the anterior edge of the coxa of the fifth limb, as is shown in fig. 3. The gland as a whole is straight, and lies midway between the lateral thickening of the entosternite (which has at this stage the same general form as in the adult) and an imaginary line dividing the body from the coxæ of the limbs. As the centre of the gland is on a level with the plate of the entosternite the two lateral ridges of that structure if they were produced as they are in Mygale would embrace the gland, a relation which, as M. Pelseneer has shown in a paper recently read before the Zoological Society, is in Mygale actually present. The

anterior end of the gland passes in front of the main mass of the entosternite, and is in relation to its anterior cornua. The blood supply of the gland could not be ascertained with certainty from the fact that all the specimens had been cut into to ensure their preservation, but from the presence of blood-corpuscles we can assert that some of the spaces in the connective tissue surrounding the gland are blood spaces, though there is no trace of the central blood-vessel which is present in the adult.

Communication with the exterior is effected by means of the primary tube, which, lying ventrally and nearest to the ento-sternite, curves gradually away from the rest of the gland, and at the level of the anterior edge of the fifth appendage is separated from it by a muscle, passes slightly downwards, turns gradually, ascends close to the main artery of the limb, passes away from this and runs for a short distance close beneath the integument, and opens at the bottom of a slit-like depression at the base of the coxa of the fifth limb on the side next the fourth appendage and on the dorsal surface. At the base of this appendage, and of the second, third, and fourth limbs as well, is a very curious sculpturing which is not present in the adult, and which can be best understood by reference to fig. 13. Furrows running in various directions separate that part of the coxa which lies nearest to the dorsal surface of the animal into three lobes, which, if looked at from the dorsal surface, lie one distal and median, and two proximal, right and left; the two proximal ones being together about double the breadth of the distal one. From the apex of the median lobe another furrow passes obliquely upwards and forwards towards the distal end of the limb, and soon bifurcates, giving rise to two right and left lobes less strongly marked than the proximal ones, the one nearest the fourth appendage being much smaller and lying more deeply than that on the side of the sixth appendage. Parallel to the coxa, and on the side of it nearest the anterior end of the body, a chitinous ridge runs, and it is at the bottom of the deep furrow between this and the smaller of the two distal lobes that the duct of the coxal gland opens.

The difference in sculpturing between the fifth and the second, third, and fourth limbs consist in the almost entire absence of the chitinous ridge in the three last, and in the fact that the distal furrows is in them nearly median in position, and the two distal lobes therefore more nearly equal in size. In the interior of the limb the furrows are represented by a thickening and slight ingrowth of the chitinous cuticle. It is worthy of note that in the adult the depression which exists at the base of the coxæ of all the limbs is most marked at the base of the fifth.

For a short distance from the opening the duct is lined by a continuation inwards of the chitinogenous cells of the integument, and these have a cuticle (fig. 11) on their internal surface, the whole being enclosed by a basement membrane, to which the trabeculæ of the connective tissue are attached. The chitinogenous cells soon pass into the proper epithelium of the gland, which is identical in structure through the whole course of the tube. It consists of a continuous layer of protoplasm surrounding the lumen, in which in a transverse section the nuclei are placed somewhat irregularly, and in which the division into cells, as in the adult, is not evident (figs. 4 to 10). The cortical part of the protoplasm when highly magnified is seen to be striated radially in the same way as in the adult (fig. 10); the internal part of the protoplasm is granular, and in it the nuclei lie. A basement membrane encloses each tube, and, as in the adult, the intertubular connective tissue is slightly modified from the ordinary connective tissue, inasmuch as the lacunæ are smaller, and the relative amount of trabecular or skeletal substance therefore greater.

The internal opening of the tube is on a level with the point where the coxa of the fifth limb is just beginning to appear in the sections (fig. 1 is at this point). The tube marked D in figs. 4 to 9 is derived, as seen in fig. 4, from tube C, and is ventrally placed alongside of, and external to, the primary tube A. Its ventral wall disappears, and for several sections its lumen is in free communication with the spaces in the connective tissue which lie be-

tween the gland and the ventral blood-sinus. It is then closed by a connective-tissue trabecula, and the tube soon disappears from the sections. The gland epithelium does not end suddenly, but gradually passes into the typical connective-tissue cells, the extent to which it passes out into the spaces varying in different sections (figs. 6, 7, 8). The basement membrane becomes continuous with the trabeculæ of the connective tissue, and on these connective-tissue cells or rather nuclei are scattered irregularly.

Note on the Foregoing.

By Professor E. RAY LANKESTER.

From the preceding observations it is clear that the coxal gland of Limulus has the essential anatomical features of a "nephridium," such as that of the Chætopod worms and of Peripatus, viz. it is, in the young animal, a tube opening to the exterior by one extremity and to the primitive body cavity or cœlom (the space between the trabeculæ of the connective tissue) by the other; further, it is a paired organ, occurring on the right and left sides of the body, and moreover the pair appear to belong to a single segment, and to be therefore possibly the single surviving pair of a number of such nephridia, of which one pair were developed originally in each segment of the body.

The conversion in Limulus of what is in the young an externally-opening tubular gland into a "ductless gland" in the adult, finds a close parallel in the history of the supra-renal body of Vertebrata as determined by Mr. Weldon (this Journal, January, 1885). The coxal gland of Limulus, with its curious brick-red pigment, is probably not only morphologically similar to the modified bit of mesonephros which forms the supra-renal body of Vertebrates, but also physiologically resembles that organ.

The observations here recorded on the structure and connections of the immature coxal gland tend to render it probable that the green glands of Crustacea (antennary coxal gland) are also to be regarded as a pair of modified nephridia. In figs. 14, 15 are reproduced Grobben's drawings of the antennary glands of two forms of Crustacea. It seems not improbable that the so-called "end-sac" of these glands is not part of the nephridium, but is developed from the connective-tissue space (cœlomic space) into which the true tubular nephridium originally opened. It certainly would be possible to bring about such a structure by allowing the space into which the inner end of the young coxal gland of Limulus opens to enlarge and become vesicular instead of allowing the nephridial tube to close up.

It is important to note further the possibility that other structures present in Arthropoda are to be regarded as modified nephridia.

The demonstration of the extensive changes which the coxal gland of Limulus undergoes in its development opens our eyes to the probability of such changes in other cases. It is a remarkable fact (as has been pointed out by Mr. Kingsley, who has independently demonstrated the tubular character and external opening of the coxal gland in the embryonic Limulus) that the "shell-gland" of Entomostraca opens at the base of the fifth pair of appendages (the second pair of maxillæ) in those animals, and thus corresponds with the coxal gland of Limulus and of the Arachnida in position. But when once the notion is admitted that ducts opening at the base of limbs in the Arthropoda are possibly, and even probably, modified nephridia, we immediately conceive the hypothesis that the genital ducts of the Arthropoda are modified nephridia.

It will require careful embryological work to test this hypothesis. It is supported by the analogy of Vertebrata, where as close a connection and as direct a continuity of the gonad with the adventitious duct derived from the renal excretory system is attained in the male sex, as is observed in both male and female among Arthropods.

The view that the genital ducts of the Arthropoda are modi-
fied nephridia is further supported by the consideration that
there is no other plausible suggestion as to their origin and
significance.   From this point of view we have to bring into
consideration all animals whose genital ducts are continuous
with the gonads and open to the exterior.   Animals are, as I
have elsewhere pointed out ('Encycl. Brit.,' article "Mollusca"),
either Schizodinic or Porodinic, that is, discharge their genital
products by rupture or by permanent pores.   The Porodinic
forms are, according to our present knowledge, divisible into
those which are nephrodinic and those which are idiodinic, the
ducts being in the first case "nephro-gonaducts," and in the
second case "idio-gonaducts."   But it seems possible that such
a thing as "idio-gonaducts" have no real existence.   The
gonad itself in Cœlomate animals is essentially a group of cells
forming part of the lining of the cœlom or body cavity, and it
seems quite likely that in all cases the duct, even when it is
intimately fused with the gonad, was primitively a nephridium.
If this is universally true we have to reckon nephridia as form-
ing gonaducts by fusion with the gonads in Echinoderms, in
Platyhelminthes, and in Nematoid worms, as well as in Arthro-
poda and Mollusca—cases which at present are regarded as
typical instances of the occurrence of idio-gonaducts.

Possibly the generalisation may not prove to be justified in
all these groups, whilst holding for the Arthropoda and
Mollusca.

The full consideration of the suggestion here made involves
a more definite conception than we at present possess of the
nephridium as a primary organ of the ancestral Cœlomate.
How many pairs of nephridia may we assign to that ancestral
animal?   Is every tubular structure opening from cœlom to
exterior necessarily to be considered as belonging to one cate-
gory—the nephridium?   How can pores such as the dorsal
pores of the Earthworm be distinguished from rudimentary
nephridia?   If pores leading from the cœlom to the exterior
have an existence independently of nephridia, how are we to
distinguish those pores which are merely "reduced" nephridia

from those which are autogenous? If autogenous pores can exist, is it not possible for the gonad to acquire continuous membranous connection with such a pore or pores, and so elaborate for itself an idio-gonaduct, which would have nothing to do with a nephridium?

These and similar questions present themselves for solution and must be answered before we can come to definite conclusions with regard to this unexplored question of the nature and significance of genital ducts.

Finally, a point of great importance, which I propose to deal with more fully elsewhere, is the fact that the space in the connective tissue into which the young nephridium opens internally is NOT a blood-space. The blood-system in the larger Arthropoda is, I have recently ascertained, altogether distinct from the general system of lacunæ of the connective tissue. These lacunæ form a "lymphatic system," which contains a liquid distinct from the blood; they represent the cœlom or body-cavity, and as such receive the internal openings of the nephridia.

EXPLANATION OF PLATE XXXVI,

Illustrating Mr. Gulland's paper on "Evidence in favour of the View that the Coxal Gland of Limulus and of other Arachnida is a Modified Nephridium."

FIG. 1.—Diagram of a transverse section of Limulus at the level of the internal opening of the coxal gland. *co. gl.* Coxal gland. *A.* Primary tube. *m.* Muscle. *h.c.* Hepatic cæca. *ento.* Entosternite. *int.* Intestine. *H.* Heart. *p.c.* Pericardium. *n.c.* Ventral nerve-cord.

FIG. 2.—Diagram of the coxal gland spread out on a plane surface. *ant.* Anterior end. *post.* Posterior end. *du.* Duct or external opening. *int. op.* Internal opening. *sep.* Septa in the lumen of the gland.

FIG. 3.—Dorsal view of young Limulus (the original was 12 millimetres in length from the anterior end to the root of the spine, 19 mm. altogether, and 12 mm. in breadth at the level of the 4th appendage, where it was broadest), to show the relations of the coxal gland. *I—VI.* The coxæ of the appendages. *co. gl.* Coxal gland. *ento.* Entosternite.

FIGS. 4—11.—Common references. *A.* Primary tube of coxal gland. *B. C. D.* Secondary tubes. *c. gl. e.* Epithelium of coxal gland. *b. m.* Basement membrane. *m.* Muscle. *c. t. c.* Connective-tissue corpuscles. *T.* Trabeculæ of connective tissue. *b. c.* Blood-corpuscles.

Fig. 4.—To show the derivation of tube D from C, and the continuity of the epithelium.

Fig. 5.—Transverse section of the coxal gland, showing the relations of tubes to one another, the intertubular connective tissue, and to the left of D, the space into which that tube is about to open.

Fig. 6.—The next section to Fig. 5; the partition between D and the connective tissue is no longer present.

Fig. 7.—The next section but one to Fig. 6, the intervening one being exceedingly like Fig. 6, was not drawn.

Fig. 8—The next section to Fig. 7.

Fig. 9.—The next section to Fig 8. The connective-tissue trabecula *T*, has closed the opening of D, which becomes smaller in each succeeding section, and soon disappears.

Fig. 10.—A small portion of the gland epithelium more highly magnified showing the radial striation of the cortical part.

Fig. 11.—Part of a section of the coxa of the fifth limb, showing the duct of the coxal gland near its opening, and while it is still lined with

chitinogenous cells. *ch. c.* Chitinogenous cells of the integument. *bm.* Basement membranes. *du.* Lumen of the duct. *b. c.* Blood-corpuscles. *cu.* Cuticle. *ctc.* Connective-tissue corpuscles.

Fɪɢ. 12.—The second to the sixth limbs of the right side of a young Limulus magnified, showing the opening of the coxal gland, *du,* in its relations to the fifth and other appendages, and also the sculpturing on the bases of the coxæ. The projecting part of the carapace has been cut away close to the bases of the coxæ. *c. c.* is the cut edge, and the animal is represented lying on its back, with the anterior end towards the left hand. The limbs are thus seen from their dorsal surface, and are numbered II to VI.

Fɪɢ. 13.—The base of the coxa of the fifth limb much more highly magnified, viewed as an opaque object under Pillischer, Obj. $\frac{5}{8}$, oc. 3, showing the sculpturing and the position of the opening, *du,* of the coxal gland. *c. r.* The chitinous ridge.

Fɪɢ. 14.—Internal termination of the antennary gland of a young Estheria (Phyllopod), showing the end-sac, *e. s.,* probably a specialised connective-tissue lacuna, and not part of the nephridium itself. (After Grohben, ' Arbeiten Zool. Inst. Wien.,' vol. iii, 1881.)

Fɪɢ. 15.—Antennary gland of Mysis. *Ceph.* Urinary tubes. *eo.* External aperture. *hb.* Urinary bladder. *e. s.* End-sac (probably a closed connective-tissue lacuna, into which the nephridium opens).
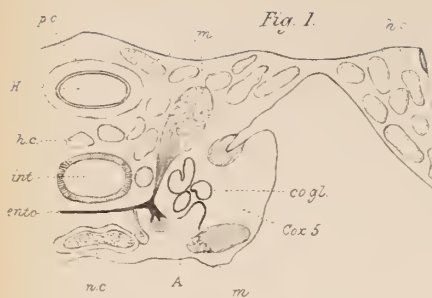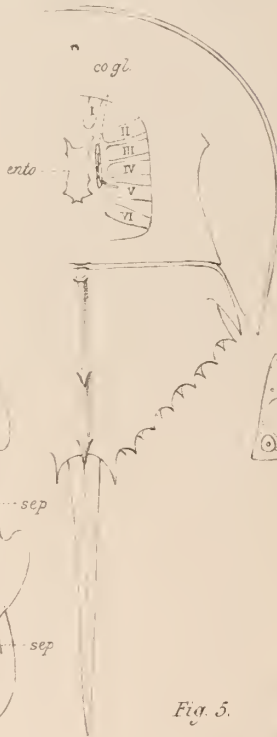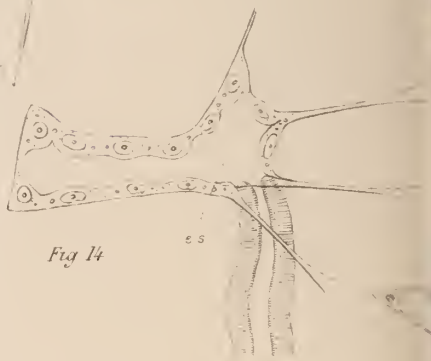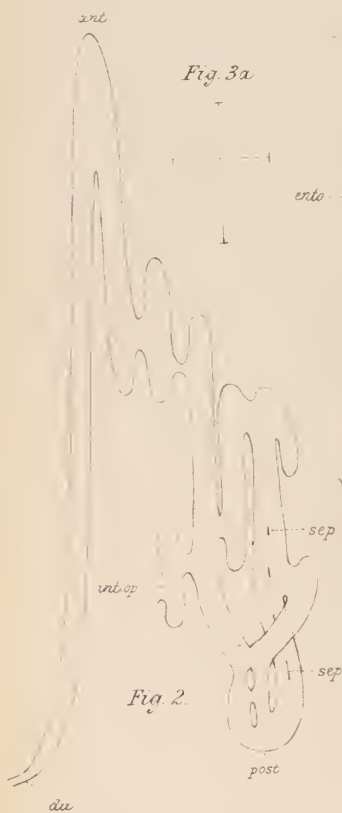
Fig. 1.

Fig. 15.

Fig. 3.

Fig. 3a

Fig. 2.

Fig. 14
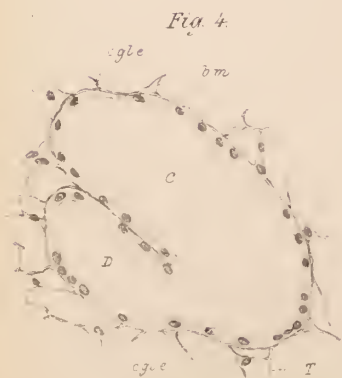
Fig. 5.
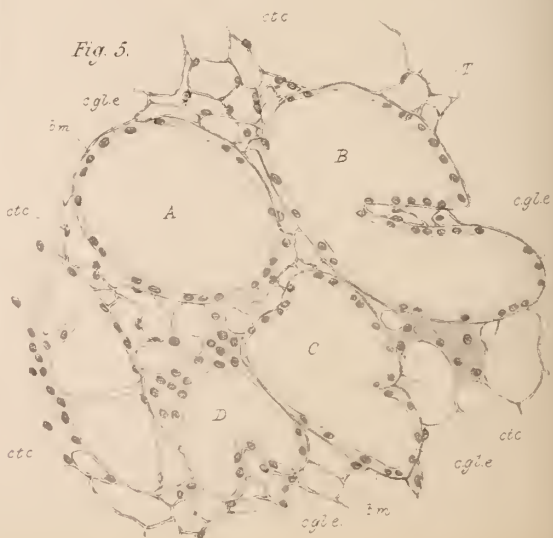
Fig. 4.

G L Gulland del

Fig 6.

Fig. 7.

Fig. 12.

Fig. 13.

Fig 11.

Fig 10

Notes on the Embryology of Limulus.

By

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With Plates XXXVII, XXXVIII, XXXIX.

SEVERAL papers have been published upon the development of Limulus polyphemus, the titles of which will be found in the appended bibliography. Since Professor Ray Lankester has recently strongly advocated the Arachnidan affinities of Limulus it has seemed especially desirable to study the embryology of this form by means of sections, a method scarcely touched by Dr. Packard, the most voluminous writer upon this subject.

I would here return my sincere thanks to my friend George H. Thompson for his assistance in obtaining material for my researches.

I have not witnessed the process of oviposition, which, however, has been described by Dr. Lockwood ('70),[1] but with some of his conclusions I would express dissent. The eggs are not all laid above high-water mark, for I have found them half way between tides, and have frequently taken the male and female coupled together, and buried in the mud below low-water mark.

All of my attempts at artificial impregnation have been unsuccessful, for animals kept in confinement, even when

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[1] I have, in referring to the bibliography, adopted the admirable plan of Dr. E. L. Mark, by which the reference number gives the reader at once the approximate date of the article. A bibliography is appended.

caught in copulating, utterly refuse to lay their eggs. I obtained an abundance of good milt, and to all appearances the eggs were ripe. Again and again was fertilization tried, but although the eggs were kept for four weeks they showed no signs of change except those that might be the effect of decomposition (see *infra*). A further difficulty was found in manipulation of the early stages of the eggs obtained from the natural nests, and many experiments were tried to ascertain the best method of procedure. For surface views a slight staining with osmic acid proved best, while for sections hardening in successive grades of alcohol proved at least equal to chromic acid, Merkel's fluid, Perenyi's fluid, or corrosive sublimate. The coagulation of the albumen by heat, so advantageous in studying many Arthropods, was here worthless. The greatest difficulty with the earlier stages was found in the extremely refractory chorion, which did not separate at all readily from the egg proper. I had in most cases to cut it and the egg together, and the results were far from satisfactory.

The eggs were embedded in paraffin by chloroform, cut in ribbons, and fastened to the slide by the collodion method. They were then stained by eosin or hæmatoxylin. They did not readily take color, usually requiring ten minutes to stain with the former dye, which generally works much more quickly. My best results were with hæmatoxylin, overstaining, and then submersing in acid alcohol.

The eggs and embryos possess great vitality, living in confinement with only the slightest care; and, as I write, I have specimens living which I obtained five months ago: they have spent the last three in a common saucer, the only attention being to replace evaporation by ordinary hydrant water. The density of the water seems to have but little effect upon them, and I have had them live for several weeks in perfectly fresh water. This vitality, which is also characteristic of the adults, has an extremely interesting aspect when we consider the fact that Gigantostraca have an ancestry extending back to the Palæozoic rocks. Professor E. S. Morse has noticed a

similar correllation between the vitality of the individual and
of the race in the case of the Brachiopods.

The eggs vary considerably in their characters.   Some are
spherical, others markedly oval.   According to my measure-
ments the average diameter is about two millimetres, varying
between 1·75 mm. and 2·2 mm.   Some are brown, some are
ashy green, and others yellow or pink.   Color seems unim-
portant, as I have had pink embryos hatch, the color persisting
for some time after escape from the membranes.   The egg is
enveloped in a thick and dense chorion, which is apparently
made up of layers, about twenty in number, the whole having
a total thickness of about $\frac{1}{1000}$th of an inch.   In rupturing
this chorion the line of tear usually goes straight across, and
but rarely can traces of lamination be seen.   Usually no traces
of pores are visible, and I have sought in vain for a micropyle.
Occasionally I have seen indistinctly what may be pores, and
it is certain, for reasons which will appear anon, that there is
some way in which water penetrates the chorion.

I greatly regret that I have nothing to offer regarding seg-
mentation and the formation of the blastoderm, but my earliest
embryos are already far along in their development.   In the
following pages I shall speak but briefly of the external de-
velopment, except when my observations are at variance with
the previously published accounts of Dohrn ('71) and Packard
('72).   In studying the internal development I am unfortunate
in having no predecessor, as I thus have nothing with which to
check my results, and hence there is more chance for error to
creep in.   Even more unfortunate is the fact that I have no
knowledge of the formation of the blastoderm wherewith to
settle some features of the later stages which are uncertain.

External Development.

The earliest egg seen was treated with osmic acid, and pre-
sented the appearance shown in fig. 4.   Upon the surface is a
longitudinal pyriform depression in the centre of a lighter field.
This lighter area corresponds to the germinal area so frequently

described in Arthropod embryology, and the depression is the so-called germinal or ventral groove. In reality, at least in the case of Limulus, the lighter area marks the extension of the mesoblast (the epiblast extending entirely around the egg), and, in my opinion, the ventral groove is a modified blastopore. To this I will recur again.

LARVAL ENVELOPES.—In this stage sections show that the epiblast cells have begun the secretion of the first larval envelopes, which Dr. Packard has endeavoured to compare with the amnion of insects. This comparison is utterly without foundation, as this is a cuticular not a cellular structure, the polygonal cell-like markings with which the surface is ornamented being due to its mode of origin. Anticipating our account a little we will trace the history of what I would call the first larval cuticle throughout its history. In the stage 4, outside the mesoblastic area, the blastoderm consists of a single layer of large polygonal epiblast cells (fig. 39) resting directly upon the yolk. Each cell is covered with a thin cuticle which refuses to take any stain. This cuticle follows closely the contour of the surface of the epiblast, extending down between the various cells, thus giving rise to the polygonal markings which, in the later stages, are confined to the external surface, and which were so puzzling to Dohrn and Packard.

At the time of the appearance of the limbs this cuticle is nearly as thick as the epiblast, having increased by additions to the inner surface. The outer still retains its markings, but the inner is nearly smooth. As yet it is in close contact with the epiblast, the cells of which are still contributing to its increase (fig. 45). When the embryo arrives at stage 11 the cuticle separates from the epiblast, and the cells of its surface have an average diameter of mm. ·0033. Of the explanation of the ensuing phenomena I am not certain, and the following is but tentative. It would seem that an osmotic action begins whereby water is taken in through the chorion and cuticle, thus creating a pressure which ruptures the

chorion.   From the time of this rupture until the assumption
of a free life this larval cuticle functions, as Dr. Packard has
aptly expressed it, as a "vicarious chorion."   As the embryo
develops this cuticular envelope increases in size, always having
a considerable space between it and the embryo (cf. Packard,
'72, pls. iv, v, figs. 19, 24, a).   This increase continues until
at last the "vicarious chorion" has about twice the original
diameter of the egg.   This increase in size is not due to any
growth, but only to elasticity.   In the later stages the pseudo-
cells are no longer visible, but long before the final hatching
they have twice their original diameter.

   A second cuticle is formed and molted before the embryo
hatches.   It makes its appearance at the stage 6, and in
fig. 11 it is represented as lifted up on the extremities of
the limbs.   The active movements of the embryo soon tear
this second cuticle off, and in the subsequent stages it is seen
as a delicate membrane wadded up at one side of the chorion.
Dr. Packard ('72, p. 165) describes this molt.

   Between 4 and 5 a considerable gap occurs in my material,
and I am not able to say whether here, as in scorpions (Metsch-
nikoff, '71) and spiders (Balfour, '80, b), any metamerism
appears before the appendages or not.   In fig. 5 all the
cephalothoracic appendages have appeared, and I have seen
no evidence to support Dohrn's idea that the first pair appears
later than the others.   Occasionally it is invisible in the living
egg, but osmic acid always renders it distinct.   An important
fact was pointed out by Packard that at this early stage all
the appendages are post-oral, although long before hatching
the first pair acquires a pre-oral position.   These appendages
are at first simple outgrowths from the surface.   In the
median line of the embryo can be seen mouth and anus, each
having a slender pyriform outline, the narrow end being in
front.   On first seeing this in connection with fig. 4, I was
forcibly reminded of Balfour's figures of Peripatus ('83, pl. xx,
figs. 34 to 37), and, were the narrower end of the mouth turned
in the opposite direction, the natural inference would be that

the blastopore had closed in the middle, leaving the mouth and anus at the ends. This, I believe, is the true explanation, allowances being made for the modified form of gastrulation (*vide* infra). The fact that the narrow extremity of the oral opening is turned forwards is due to its being in the act of transference from a position in front of the first pair of appendages to one behind it. Between the mouth and anus is apparently a shallow groove. In reality this is produced by the neurulation, and is internal. Sections show that the external surface is smooth (fig. 47), and that the blastopore has entirely disappeared, except as mouth and anus.

The first pair of abdominal appendages (opercula) are partially marked out, and it is to be noticed that these and the gill-bearing appendages develop in a slightly different manner from the true limbs, a fact which would not be inferred from Dr. Packard's drawings (e. g. '72, pl. iv, fig. 16). In reality they arise as broad plates, separated from the surrounding surface by a tucking in of the epiblast behind (cf. ' Formation of Lungs of Scorpion,' Metschnikoff, '71, p. 225, pl. xvi, fig. 12). Besides the opercula the abdomen as yet shows no trace of segmentation or appendages. The limit of the mesoblast is much more distinct than in the preceding stage. It extends slightly beyond the limbs, and has an elongate oval outline slightly narrower in front. Outside the limits of the mesoblast, on a line between the fourth and fifth pairs of appendages, are seen the rudiments of the compound eyes. They are not visible in the living egg, but stain readily and deeply with osmic acid.

Between this and the stage figured in 6 the changes are slight. This is slightly earlier than Dr. Packard's ('72) pl. iv, fig. 16. The mouth and anus still retain the same outlines as before, and the neural groove is still conspicuous. The limbs are now elevated above the surrounding surface and begin to show traces of flexure. In the abdominal region the opercula are better developed and the first pair of gill-bearing appendages have appeared. In this and earlier stages I have failed to find that segmentation outside the mesoblastic area

described and figured by Dr. Packard as existing outside the
" germinal area," and am of the opinion that it does not
exist, as usually metameric segmentation does not exhibit
itself in epiblast alone, and the mesoblast has not yet extended
itself so far.

As was noticed by Dr. Packard, between this and the next
stage a second moult takes place, the cuticle being lifted up upon
the feet as shown in fig. 11. Now motion begins, and the
movement of the limbs, at first very slow, tears this second
cuticle, the remains of which can be seen until the time of
hatching inside the " vicarious chorion."

In the next stage necessary to be mentioned (fig. 12) a limu-
loid appearance is visible. The body is elongated and the
abdomen is differentiated, and it and the cephalothorax are dis-
tinctly segmented, six somites being visible in the latter and
eight in the abdomen, the ninth or telson not being separated
from the preceding segment. The cephalothoracic limbs are
jointed and the chelæ outlined, while the peculiar whorl of
spines on the sixth pair is already formed. The flabelliform
appendage on the outer side of the coxa of the same pair and
the metastoma are also visible. In the abdomen the oper-
culum and the first branchial appendages are well developed,
and the gills upon it are beginning to be formed. The second
branchial appendage is just appearing.

Fig. 14 corresponds so closely with Dr. Packard's ('72) fig. 24
that there is no necessity for describing it here. I would, how-
ever, state that his representation of the appendages (a matter
of no great morphological importance) is extremely faulty, and
in 24, a, the eye is much too remote from the margin. At this
stage specimens just killed with alcohol show the posterior
portion of the nervous system very plainly through the integu-
ment, but I have not been able by surface views to show
exactly the innervation of the anterior pair of limbs. This,
however, is to be the less regretted, as my sections settle this
point. I would in passing state that Dr. Packard's ('80, a) pl.
v, fig. 8, cannot be relied upon as proving that the first pair
are post-oral ; the stage is too late for that demonstration.

Figs. 16 and 17 correspond with Packard's ('72) figs. 25 and 25, *a*, reproduced here. They represent the young Limulus as it leaves the egg. It is 4 mm. long; the cephalo-

Limulus just hatched.

thorax has a semicircular outline, and the abdomen, though smaller, has a similar shape. The dorsal surface of the cephalothorax is without traces of segmentation except in the lines bounding the cardiac region, where the depression marking the attachment of the muscles of the limbs are visible. These are not shown in the adjacent woodcut taken from Dr. Packard's paper, which is otherwise good, except that the segmentation of the abdomen is much less distinct in nature. I may say here that the segmentation in Limulus nowhere affects the epiblast and its derivatives, and at no time in the development do we find the body divided into a series of somites moveable upon each other like those in the abdomen of a lobster; the only joints are those between the cephalothorax and abdomen, and between the latter and the caudal spine.

Dr. Packard's figure of the ventral surface at this stage is very inaccurate. A better illustration will be seen in fig. 17, from which it will be seen that the appendages are closely similar to those of the adult female.

A number of individuals which hatched on the same day were isolated and the first moult after hatching was watched for with considerable interest. Soon after hatching the young Limuli bury themselves in the sand as do the adults, and appa-

rently undergo exuviation while thus concealed. The carapax
splits along the frontal margin as in the adult. The first
specimen moulted in exactly five weeks after hatching, while
the others struggled along at intervals of one or two days, the
last one of the lot casting its skin in seventy-two days after
leaving the " vicarious chorion." After the moult the animal is
much larger, and the most important change is in the increased
length of the caudal spine, the general appearance being shown
in Packard's ('72) fig. 27 of plate v. For our purpose it is not
necessary to carry the account of the external changes any
further. Nowhere in the external development are there any
startling metamorphoses, and nowhere, except in the closure of
the blastopore and the post-oral position of the first pair of
appendages, do we find facts which aid us in a discussion of
the affinities or phylogeny of the Xiphosura ; certainly nothing
which would indicate Crustacean relationships.

INTERNAL DEVELOPMENT.

The earliest stage of which I possess sections is that of fig. 4,
but from difficulties of manipulation these are not very satis-
factory, though they show some important points. The egg was
cut transversely to the rudimentary blastopore, and one section
is shown in fig. 44. The epiblast, a single cell in thickness, enve-
lops the whole egg.   The groove, which I regard as blastopore,
is as deep as the thickness of the epiblast.   Beneath this groove
and extending to a short distance either side is the mesoblast,
the limits of which correspond to the lighter area in fig. 4.
This mesoblast arises wholly from the bottom and edges of the
groove, and in the sections a rapid cell-proliferation is visible
here.   Beneath these two layers (epiblast and mesoblast) is the
yolk.   I believe that not only this groove but the primitive
streak of all tracheates, as suggested by Balfour, is the homo-
logue of the blastopore and that the yolk is wholly hypoblast.
Schimkewitch ('84) comes to the same conclusions from studies
of the spiders.   How this modification arose I am not now
ready to discuss, but when we consider the recent researches
of Balfour on Peripatus, Hatschek on Amphioxus, and

Bateson on Balanoglossus, and especially the Hertwigs' Cœ-
lomtheorie and Sedgwick's essay on metameric segmentation,
and the light they throw on the origin of the mesoblast, I
think we are justified in reversing the course of reasoning and
concluding that, on account of the origin of the mesoblast, the
primitive groove is the homologue of the blastopore.

The hypoblast has recently been recognised in the yolk,
though in a rather vague and indefinite manner. Only one
recent author (Ayres, '84), so far as I am aware, has had other
views. The indecision has doubtless been caused by the late
appearance of the archenteric, or rather mesenteric lumen.
To this we will recur again; but now I call attention to the
fact that at the stages of figs. 4 and 5 the yolk is broken up into
true cells, each with its nucleus and cell wall. I have yet to
see any "free yolk-nuclei." In describing the development
of spiders Balfour ('80[b]) refers to and figures cells migrating
from the yolk and taking part in the formation of the meso-
blast, and says that the middle part of the dorsal mesoblast
arises largely, if not wholly, in this manner. On the other
hand Patten ('84), treating of the development of the Phry-
ganids, reverses the operation, and claims a migration of meso-
blast into the yolk. My observations do not allow me to
decide which of the two is correct; indeed, I have seen but
very slight indications of any migration, and those would, as I
interpret them, tend to show that part of the mesoblast which
forms the heart may arise in this manner. This view (a
migration of yolk-cells into the mesoblast) does not conflict
with the more recent ideas of the origin of the mesoblast in
triploblastic animals nor with the nature of the yolk as above
expressed, but merely shows that it is archenteric rather than
strictly hypoblastic. Mr. Patten's views are rather difficult to
reconcile with what we know of the origin of the mesenteric
tissues in other Arthropods. It would seem to me that he
has misinterpreted his facts.

Of the closure of the blastopore or neural groove I have
nothing to say, as I have seen no eggs between figs. 4 and 5.
Still, judging from the appearance of the two, I think the

inference justifiable that here, as in Peripatus capensis,[1] it closes in the middle, the extremities persisting as mouth and anus.

On a previous page I spoke of the shape of the oral opening, and said that it was due to the transference of the mouth. The mouth in fig. 5 is in front of the first pair of appendages, in the adult behind them. The process of this transference is interesting. After the disappearance of the primitive groove behind the mouth a depression gradually extends backwards, and at the same time at the front the edges rise up and finally unite to form a close tube, the stomodæum (figs. 40, 41, 42, 43), in almost exactly the same way that the neural canal is formed in the chick. This seems to me an important point, for it shows that the functional mouth is not a strictly homologous structure throughout the animal kingdom, but that in those forms with a stomodeum it has been considerably modified in position. Unfortunately we do not know if a similar modification exists in Peripatus. The anus (fig. 46) is a shallow pit, and at this stage shows no signs of forming a proctodæum.

From this point on it will prove the easiest and possibly the best to consider separately each of the tissues or organs without attempting to describe the embryo at each stage as a whole.

MESOBLAST.

In fig. 4 the mesoblast constitutes a broad sheet (fig. 44), but between this and fig. 5 a considerable gap occurs in my material. In this latter stage it has become separated into two broad bands, except at the extremities. It seems probable that this separation is effected partly by the rapid growth of the epiblast in the ventral region, and partly by a migratory movement of the cells. In the region of the mouth it is still

[1] Kennel's ('84) recent researches on two South American species of Peripatus do not prove Balfour wrong in his interpretation, and until further observations are published on P. capensis, I think that his observations, certified to by Moseley and Sedgwick, should be accepted. Nor do I think Kennel has proved Sedgwick's ideas "ungeheurlich," for Lang on the Planarians and Wilson on the Alcyonaria come to the same general conclusions.

continuous and not differentiated from the epiblast, although it has become separated in the anal region (fig. 46). In the middle region the broad bands on either side extend into the legs (fig. 47), but no trace of a cœlom has yet appeared. On the neural side of the limbs it is a single cell thick, but in the region of the appendages it is much thicker. This thicker portion soon splits into somatoplure and splanchnoplure, and the resulting cœlom extends into the legs (fig. 30). It is to be noticed that the cœlomic cavities are separate, one being formed to each segment on each side, and further, that each metameric cavity forms at first as several parts which afterwards unite. In the stage of fig. 6 the mesoblast has extended itself to the edge of the carapax where it thins out, the cœlom not reaching quite so far. In a slightly later stage (fig. 34), but still not far enough advanced to be equal to fig. 11, longitudinal sections show larger cœlomic cavities, eight in number, one for each segment developed.

The mesoblast gradually extends itself upwards on either side until at the stage of fig. 12 (fig. 21) it meets as a single layer of cells in the dorsal region. On the dorso-lateral region it forms a longitudinal band-like thickening (fig. 21, *m*), the earliest appearance of the extensor muscles and the points of attachment of the muscles of the limbs. At the same time on the ventral surface of the body, either side of the median line, portions of the mesoblast grow up into the yolk, dividing it into segments (fig. 22, *mp.*). By this segmentation, as shown in the section just referred to, we have conclusive evidence that the metastoma (chilaria) is not to be regarded as a morphological appendage, since both it and the sixth pair of legs arise from the same segment. This was more than suspected by Professor Lankester, and is an important point in the series of homologies he has suggested between Limulus and the Scorpions. I might incidentally mention that embryology affords not the slightest evidence of the missing abdominal segments needed to render the correspondence between the two exact.

These septa not only furnish the boundaries between the segments, but they also give rise to the muscles of the appen-

dages, and eventually join the dorsal mesoblastic bands mentioned a few lines above. The septa are not wholly formed from these ventral ingrowths, but at the same time lateral inpushings are taking place as shown in fig. 13, the section being horizontal and passing above the level of the eyes, so that only a few of the abdominal segments are included. The result of this is that the yolk in the cephalothorax is broken up into a central mass and six pairs of lateral lobes, the history of which will be traced later.

It is not an easy task to trace the history of the cœlom past the point where we left it, though some isolated features are readily seen. Thus it is seen that the mesoblast does not split in the dorsal region until after the formation of the heart as a tubular organ.

Heart.

Soon after the union of the two halves of the mesoblast in the dorsal region a longitudinal cord several cells in thickness is formed. How this thickening is produced I cannot say. In the yolk beneath it the nuclei are very numerous, and the cells are much smaller than in other parts, and it may be that some of these migrate into the mesoblastic tissues ; but although I have examined many sections I have not yet seen any indisputable evidence of such migration.

Soon a lumen appears in this cord, and the size increases, at least to a considerable extent, through the budding of mesoblast cells into the tube, where they become transformed into blood-corpuscles. These processes are represented in figs. 31 to 33, which represent sections from the same individual, 31 being the most posterior. Two sections back of this is the end of the lumen. Between 31 and 32 intervenes a distance of 0·025 mm., and between 32 and 33 a distance of 0·2 mm. From this it appears that the heart is formed from in front backwards, and gradually the walls are reduced to a single cell in thickness. Not until after this stage is reached does the heart separate from either of the mesoblastic layers, and from these, first from the splanchnoplure (fig. 33). This single-celled

condition of the walls is found in all the embryonic stages, and the heart does not have epithelial and muscular layers until after hatching. This mode of the formation of the heart is paralleled in both Crustaceans and Spiders, and hence throws no light upon the affinities of Limulus.

At about the same time that the dorsal mesoblast begins to thicken for the heart a similar thickening is noticeable in the epiblast immediately above it (fig. 31). Reichenbach ('77) has noticed a similar thickening in the Decapods. Of the meaning I am uncertain; it may be that it is the remains of a degenerate " dorsal organ," but it seems more probable (at least in the case of Limulus) that it is the early stage of the median dorsal crest or ridge of the adult. In the latest stages which I have studied I have found no further change in it.

Segmental Organs (nephridia).

Under this head I would place the brick-red glands first noticed by Dr. Packard ('75ᵃ), and recently described in detail by Professor Lankester ('84). Concerning their earliest stages I am yet in doubt. The earliest trace of them which I have seen was in the shape of two patches of mesoblast, one on either side in the fifth segment of the body. With growth they increase in size, forming a well-defined tube, and join the epiblast by the posterior extremity. This junction takes place in the posterior coxo-sternal articulation of the fifth pair of legs, and soon after an opening appears enabling the organ to communicate with the exterior. I have not been able to follow exactly the way in which the complicated organ of the adult is developed from its comparatively simple beginning, as I have had to rely solely on sections. From these (figs. 23, 26, 27, and 30) and others I have constructed a diagrammatic figure of the shape of the organ at the time of communication with the exterior (figs. 9 and 10). From the opening a narrow tube lined with quadrate epithelial cells goes forward and upwards (fig. 23) a short distance and then widens out into a spacious sac, which narrows again before reaching the fourth segment of the body. The cells of this portion are more columnar, and

have the nuclei placed at the ends away from the lumen.
From this point the tube bends back on itself, going back a
short distance, and then turning again enters the fourth
segment, where it turns again and comes back to its first turn,
where it terminates.   As to the character of the termination I
am yet in doubt, although I have examined many sections,
both transverse and longitudinal.   In some it appears to com-
municate directly with the body cavity, the internal end being
open (fig. 27, *fn.*).   I have been unable to detect cilia in any part.
In various parts of the tube the epithelium varies in character
between columnar and quadrate cells.   In all the cells the
nuclei are very large, and are placed at times nearer the fore, at
others to the deeper end of the cells.   The general character of
the quadrate cells is shown in fig. 28, which closely resembles
Professor Lankester's delineations of these glands in the adult
Limulus.

I see no reason why these glands in Limulus and the corre-
sponding ones in the Scorpion, together with the so-called
shell-glands of the Crustacea, should not be regarded as seg-
mental organs.   Later in this paper I will return to their
discussion, but here I would call attention to the fact that at
least in the later stages the inner end of the gland terminates
cæcally as in the various Crustaceans.   The closure of its
efferent duct takes place later.

Respiratory Organs.

On a previous page I have described the early stages of the
abdominal appendages.   They arise as broad lamellar out-
growths from the lower surface of the abdomen.   At first, and
in fact until the appearance of the gill books but two of these
appendages are visible.   These correspond to the operculum
and first gill-bearing appendage of the adult.   The others arise
in regular sequence until the whole number (five) is reached.
At first each of these appendages is simple, nothing that could
be interpreted as a biramous condition appearing until the
stage of fig. 12.   One fact requires mention here : these appen-
dages are from the beginning broad and leaflike, differing in

this respect from the corresponding embryonic limbs of Arachnids.

The operculum never develops gills, but in the adult bears the genital openings.   In the others the leaflike respiratory organs first appear at the stage of fig. 14.   The method is shown in figs. 37 and 38, and needs but little description.   The leaves of the gill book arise as outgrowths from the posterior surface of the appendage, accompanied apparently by an intucking of the adjacent epiblast.   This operation takes place first at the distal portion of the appendage, and new leaves are added at the base, the whole series overlapping each other like the shingles on a roof.

So far as I am aware, Professor Van Beneden was the first to suggest ('71) the homology between the branchiæ of Limulus and the pulmonary books of the Arachnids.   This was further elaborated by Professor Lankester ('81).   At first this resemblance seemed as far-fetched to me as it did to Dr. Packard ('82, p. 290), but subsequent studies seem to me to indicate its general validity, although I am not ready to follow all of Professor Lankester's intermediate steps, nor those of McLeod ('82).

Of the development of the pulmonary organs in the Arachnids the literature is extremely scanty, but with Lankester I am inclined to believe that when more is known of it, it will be found that the lamellæ arise in connection with the temporary abdominal appendages.   On this point Metschnikoff, treating of the Scorpion, says ('71, p. 225): "The lungs also arise as invaginations of the epiblast (Hornblattes), which appear close under the segmental appendages of the four abdominal segments (Taf. xvi, fig. 12, pn).[1]   They appear from the first as pocket-like sacs, which open by a broad mouth.   With the further development of the lung sacs, which is accompanied by an atrophy of the abdominal segments [? appendages] (with the exception of the second pair of the same), they become more spacious and deeper.   Only at the latest embryonic stage (Taf. xvi, fig. 14 from the ventral, fig. 15 from the dorsal, sur-

[1] Reproduced on Pl. XXXVII, fig. 15.

face) there grows from the dorsal wall of the lung a blind pro-
jection (Auslaufer), by which the leaf-formation of the interior
of the pulmonary cavity is begun.   The external opening is at
this time evidently smaller.   The walls of the embryonic lungs
are lined with cylindrical epithelium, which secretes a thin
cuticula.   On the outer upper surface of the lung occur here
and there some cell masses, which belong to the middle layer."

Bertkau ('72, pp. 211, 212), speaking of the increase in size
of the lungs of Lycosa, says : "Mit dem Wachsthum der Spinne
wächst auch der Luftsack und zwar stärker als das Stigma, so
das seine Spitze bald weit von demselben entfernt ist.   Der
erste Anlage eines Fächers zeigt sich in Auftreibungen
des Bodens des Luftsackes, von diesen die jedesmalig jüngste
dicht neben der nächst älteren entsteht und durch Intussus-
ception neuen Bildungsmaterials wächst."

Both of these accounts, as far as they go, agree with the
development as it occurs in Limulus, and the addition of new
lamellæ, as described and figured by Bertkau, is exactly paral-
leled by that occurring in Limulus, both in position and in
manner, if we accept Lankester's views or those given below.

It, however, seems to me hardly necessary to invoke the aid
of "parabranchial stigmata" in order to derive the internal or-
gans of the Arachnids from the gills of Limulus.   The change
in the needs of the animal owing to its assumption of a terre-
strial in place of an aquatic life would seem to be sufficient to
account for a part.   Such a change in habit would necessitate
a protection for the respiratory organs, and this would be best
secured by an outgrowth of the surrounding parts, so that
finally appendages and gills would be enclosed in pits, the open-
ings of which would then grow smaller.   The greatest difficulty
with this whole homology, as carried out by Lankester, is that
this opening must become completely closed, and another one
developed, that the side which at first was exposed to water
and then to air should subsequently be bathed with blood,
while the portion which originally contained the vascular fluid
should finally become opened to the air.   Although I believe
that there is a genetic relationship between the respiratory

organs in the two groups this physiological change is too great
to be readily accepted until we know more about the develop-
ment of these organs in the Arachnids.   Still, the description of
Metschnikoff quoted above is, so far as it goes, not wholly in-
compatible with this view, that the primary stigma formed by
the insinking of the respiratory book is not the functional one
of the adult, since this author notices its decrease in size, while
the mode of origin of the lamellæ from the dorsal surface of
the cavity is still more in its favor.   Emerton ('72, pl. 2, figs.
11, 13, 15) represents the abdominal appendages of the embryo
Pholcus as broad and like those of Limulus at a corresponding
stage, a fact opposed to their being merely modified ambula-
tory appendages, but in full accord with their homology with
those of Limulus.

On the other hand, the derivation might be much more
direct, and thus avoid the inversions and the functional
changes.   As I have mentioned above, the process of formation
of the gill-leaves is largely by a process of outgrowth, but there
is also a slight ingrowth, especially noticeable at the distal
portion of the appendage.   This, however, disappears with
growth, but is very noticeable in all my sections.   To transform
the gill of Limulus into the lung of Scorpio it is only necessary
that, together with the sinking of the whole organ, as described
above, the inpushings of the integument to form the lamellæ
should be exaggerated, and the outgrowths correspondingly
decreased.   On Plate XXXVII, figs. 18 to 20, I have diagram-
matically illustrated the steps in the process, the gill-leaves
being few in number to secure clearness.   In 18 we have the
typical condition found in Limulus, one appendage being shown
half in section and half in perspective.   In 19 we have an in-
termediate condition, when, as suggested above, the animal
was leaving the water and seeking a terrestrial life.   Here the
gill-bearing appendage (*ga*.) is partially sunk in the surround-
ing tissues to secure protection.   The same causes would also
tend to produce a similar change in the gill-leaves (*gl*.), and
they would also tend to be formed rather as ingrowths than as
protruding processes.   This change in structure would be the

more readily effected on account of a change of the medium of respiration. A gill needs either to project freely into the water, or to have some means of constantly changing the fluid which bathes it. An organ for aërial respiration, on the other hand, is not so restricted in its position, since the air is more fluid and more elastic, and hence more readily changed. Another advantage to the animal resulting from the change is that the oxygen is thus· brought nearer to the tissues requiring it.

In fig. 20 we have a diagrammatic representation of the pulmonary sac of the Arachnids. The appendage (*ga.*) has now become sunk in the body and the hole through which it passed is the stigma (*stg.*) The gill lamellæ have entirely disappeared and the pulmonary ones (*pl.*) have taken their place. The process here described is different from that imagined by McLeod ('82). It accords more with the development of the gills in Limulus, and avoids the necessity of union of the gill-laminæ and the expansion of the sternum.

Having derived the lungs of the Scorpion in this manner, but little needs to be said concerning the origin of the tracheæ in the spiders. Many years ago Leuckart showed that the so-called lungs of the Arachnids were but modifications of the peculiar tracheæ of the same group. This conclusion holds good to-day, and I would accept it in an inverted condition : The tracheæ of the Arachnids are but modifications of the pulmonary organs existing in some of the group. To transform the lungs into the other type of organ but slight changes are necessary. A prolongation of one of the sac-like pulmonary lamellæ towards the thorax gives the condition found in Argyroneta; a slight amount of branching produces the tracheal system of Zilla, and so on through forms like Thomisus until the most complicated condition is reached. McLeod's observations are interesting in this connection.

The existence of the so-called spiral threads in the tracheæ of some of the Arachnids is to be explained on mechanical grounds. In some forms nothing of the sort is found and here the tracheæ are flattened tubes. To prevent them from being

completely closed by the pressure of the surrounding viscera and to enable them to open readily for the inspiration of air on the relaxation of the pressure some elastic element is needed. This is supplied at first by scattered chitinous rods or thickenings; next, these are arranged transversely to the tube, and, lastly, the rods become united to form the spiral. All of these stages can be seen in the adult Araneinæ. Leydig's observations ('78, p. 265) on the respiratory apparatus of the Oniscidæ are suggestive in connection with the origin of tracheæ. They will be referred to again.

Nervous System.

Not until the mesoblast has become divided into the two bands mentioned above do we find any trace of the nervous system. Its first appearance is as two longitudinal epiblastic thickenings (fig. 47), one on either side of the median line. There is no external neural groove, but in its stead one on the inner surface of the cord. This of course is a variation from the normal of but minor importance, and doubtless arises from the fact that the egg fills its envelope so completely that an inward bending is impossible. In either band the arrangement of the cells show that a rapid proliferation is taking place, the result being a broad band on either side, the inner portions of which are to form the neural cord. At first, as is shown by longitudinal sections, this neurulation is continuous, there being no differentiation into commissures (connectives)[1] and ganglia until a later date.

The separation of the neural cords from the epiblast takes place at first in front and progresses more and more slowly posteriorly (fig. 29). The separation of the commissural portions is effected before that of the ganglionic areas, and in the latter the cleavage takes place first in the median line (fig. 25), even while in the lateral areas the epiblast is still

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[1] I follow Professor Lankester and others in restricting the use of the term commissure to the cords whcih serve to unite the ganglia of the same pair, and employing for those which unite the ganglia of a side the term connective.

adding to the substance of the nervous system. I am not positive as to the origin of the commissures. From the figure last cited it would seem that here as in many other Arthropods they arose as epiblastic ingrowths. This at least is most reasonable. In the stage of fig. 12 (fig. 29) there are distinctly eight pairs of postoral ganglia and this number is not exceeded in embryonic life. Of these six correspond to the six pairs of ambulatory appendages, one to the operculum and one to the first pair of respiratory appendages. As will be seen, there is no ganglion for the metastoma (chilaria), another proof, if more were necessary, that they are not to be regarded as morphological equivalents of the limbs.

In the stage of fig. 14 several important features are introduced. Here begins that concentration of the anterior part of the nervous system which results in the nervous collar of the adult. I have found it difficult to trace the changes and have obtained my best results by external views of the whole animal treated in the following manner:—On removing the embryo from its envelope I placed it in a watch crystal on the stage of the microscope and then added some fifty per cent. alcohol. This, on penetrating the cuticle, first affected appreciably the protoplasm of the nervous centres rendering it alabaster white, and thus in most regions readily distinguishable against the darker background formed by the as yet indifferentiated food yolk. Soon, however, other parts were affected and their distinctness was lost. Fig. 14 was drawn from these specimens so treated, and as a camera was used it is correct in all respects except possibly the brain and the metastoma. These parts I was unable to see distinctly, and from my sections I am inclined to believe them incorrect. This figure shows posteriorly the nerves going from the respective ganglia to the sixth, fifth, and fourth pairs of legs. In the third and second pairs of nerves there seems to be a shifting forwards so that they do not arise exactly opposite to the centres of the ganglia but rather from their anterior margins. I have shown on a preceding page the manner of the shifting of the mouth, and this change in its position, and, consequently, in that of the limbs

accords well with the facts just noted. The anterior appendages were thus brought further from their nerve-centres and hence (if I may use the expression) they exert a tractive force first on the nerves which supply them and secondarily on the ganglia, for it is for the evident advantage of the animal to have the nerve-centres nearer the parts most used.

The foregoing account mentions five postoral and pregenital ganglia; the remaining one, the first of the series, has, as far as I can see, been merged in the œsophageal collar, and the corresponding pair of nerves (which supply the small first pair) appear to arise from the outer surface of the collar at about a level with the posterior margin of the brain. Of the exact position, however, I am not quite certain.

I have spoken above of the commissures, and while not sure of their origin, I am confident that the connectives do not arise as secondary epiblastic invaginations, but merely as differentiations of the primary neural ridges. With the specialization of the ganglia, which is brought about by a more rapid increase in size, the formation of the fibrous portion of the nervous system begins. In transverse section this has a granular appearance with scattered superficial nuclei, which may play a part in the formation of the neurilemma. The nerves to the appendages arise as outgrowths from the ganglia which extend themselves among the mesoblastic tissues (fig. 35). These outgrowths contain but few nuclei at any stage but are mostly fibrous, and are directly connected with the corresponding portion of the central nervous system.

One of the most marked peculiarities of the adult Limulus is the fact that the ventral nervous cord is ensheathed by the large sternal artery, a fact without parallel except in the Arachnids. The early stage of this artery is shown in fig. 36. At all times there is a considerable space between the mesoblast and the nervous system, and at this time processes begin to grow out above and below, on either side, from the mesoblast. These in the section figured partially embrace the cords; at a later stage the two halves unite and form the walls of the artery.

The description of the development of the brain, of the eyes, the midgut and its appendages, the genital organs and other mesoblastic structures, I leave for the second part of this paper where will be discussed their bearings. Still a few facts may be of interest here, without the details which will be given later.

The brain is at first separate from the rest of the nervous system. It arises as two halves and each lobe is divided in front as shown in fig. 11, presenting a marked similarity to that of the spiders. The mesenteron is formed from the central mass of yolk, the lumen appearing after the first moult after hatching. The diverticula of the liver arise from the lateral yolk masses, and the primary lobes of which it is composed are produced by the mesoblastic septa of the cephalo-thoracic region (fig. 13). After the stage where we left the œsophagus on a preceding page, it continues to elongate and joins the midgut soon after the lumen in the latter begins to appear. It is at all times much longer than the proctodæum. The outer layer of the cells of the mesenteron feed upon the central ones, absorbing and assimilating them, thus producing the lumen.

The Position of Limulus.

Professor Lankester has so recently discussed in an able manner the relationship of Limulus ('81) that all that I can add are the few facts gained from embryology. I must admit that at first I was strongly inclined to regard Limulus as a Crustacean, but a careful consideration of the subject leads me to believe in its being much more closely allied to the Spiders, and its being a representative in the seas of to-day of the stock from which the Scorpions sprang. On the other hand, its relationships to the Phyllopods are marked; in fact, it takes us back to a time when the distinctions between the Crustacea and the Arachnida were far less marked than they are to-day. The day of a belief in "types" is past, and yet some of the terminology once in vogue is convenient; in this way we may still call Limulus a synthetic type.

Since writing the earlier part of this paper I have learned
that during the past summer Mr. H. L. Osborn attempted
artificial fertilization of the eggs of Limulus at Beaufort, and
that like myself he came to the conclusion that the operation
was not a success and threw these eggs away. A few, how-
ever, were overlooked, and when found some time afterwards
it was seen that they were really developing, and that the
early changes were very slow. This fact recalled at once my
observations on the eggs which I attempted to fertilize and the
early changes that I witnessed in them, which I thought to be
the indications of decomposition. At first the eggs were
regularly granular, but half an hour after impregnation the
surface inside the chorion became irregular and pitted with
cavities varying greatly in size, each filled with a transparent
fluid, which at the time I interpreted as caused by a migration
of the protoplasm to the surface (fig. 1). These pits in-
creased in size and number especially at one side of the egg,
until they ran together leaving the surface ornamented by a
number of hemispherical globules of yolk which twenty-one
hours after impregnation presented the appearance shown in
fig. 2. After this, though I kept the eggs for several weeks, I
noticed no change except in those which were undoubtedly
spoiled except in one instance. In that egg, twenty-nine hours
after impregnation, a profile view showed two mushroom-shaped
bodies (fig. 3) raised from the rest of the yolk, and suggest-
ing polar globules but not very vividly. The meaning of these
three observations is uncertain. If connected with the deve-
lopment of the egg the second would at once recall the peculiar
segmentation occurring in some spiders. It is not certain
that it was a normal condition, and any conclusions drawn
from it are of no value until it is confirmed.

The first definite fact is the formation of a larval cuticle.
This Dr. Packard regarded as cellular, but as he used no
sections his mistake, as well as that of Dohrn in regard to the
same envelope, is readily explained. Since the publication of
my preliminary note Dr. Packard has re-examined the subject,
and informs me that he agrees with my account of the nature

of this envelope.   This early moulting of a cuticle seems to me to be of but little importance in ascertaining the relationships of Limulus, for it is paralleled more or less completely in both Crustacea and Arachnida.   The cases which will at once suggest themselves are those of Apus, as described by Zaddach ('41), and Atax, by Claparède ('68).   Indeed, it might be well to adopt the latter author's term deutovum for all such envelopes.   Other cases, where the correspondence is not so exact (Asellus, &c.), will readily be recalled; but until a more definite knowledge is obtained of the exact origin of these deutova speculation as to their homology and meaning is useless.   Kennel's speculations ('84) seem poorly founded.

The simultaneous appearance of the six pairs of ambulatory limbs seems equally unimportant, paralleled as it is with more or less exactness in either group.   It merely indicates a concentration of development, and the fact that through abundant food supply the embryos or its nearer ancestors have not been compelled to begin free life at an early stage.   The closest resemblance, however, exists in the scorpions and spiders, where, the first pair of appendages excepted, the corresponding parts appear.   Were Dohrn's account true nothing more could be asked, but as I have had abundant material of earlier stages than any of his I believe that (as he suggests) he overlooked the first pair.   I have not seen the slightest evidence in favour of his account.   There is nothing in the development of Limulus that even suggests a Nauplius or a Zoea.

The position and early appearance of the compound eyes suggest some points of interest.   Since in the Decapod and some other crustacea (Squilla, Branchipus, Tanais, &c.) the compound eyes are borne on stalks, which are articulated to the body, some morphologists have adopted the idea that these pedicles are homodynamous with the true limbs, and a few have even gone so far as to seek an " ocular segment " in the head of Hexapods.   Without entering into a discussion of the many arguments against this view (which I believe totally erroneous even in the case of Squilla), I would say that I regard the eyes of all Arthropods merely as specialised portions

of the epiblast of the head,[1] and as having a common phylo-
genetic origin, namely, from an Annelid ancestor.

Such being the case, I regard the dorsal surface of the
cephalothorax of Limulus as but the greatly expanded upper
portion of the head, and believe that the segments indicated by
the six pairs of appendages below are without proper terga.
If we follow Packard and Lankester and recognise the dorsal
surface of the shield as composed of six (or seven) coalesced
terga, then the fact that the eyes are borne on the fifth or
fourth and fifth[2] of these segments is difficult of explanation.
This vagueness of expression as to the position of the eyes results
from the fact that the dorsal epiblast of the cephalothorax
never segments or shows any traces of division into somites,
while in different specimens the boundary between the fourth
and fifth mesoblastic somites is not constant in position as
regards the eye, but at times it passes in front of the eye and
at others beneath this organ, so that half of it is in one seg-
ment and half over the other. The ocelli are placed over the
first of these segments. It seems to me that these facts can
be explained only in the way indicated at the beginning of this
paragraph.

A nearly parallel case is found in the carapax of the Decapod
Crustacea. As usually described the carapax of the lobster or
crayfish is regarded as composed of the coalesced terga of the
segments visible below, and the obliquely transverse section
which crosses it (the " cervical suture ") is held to indicate the
line of division between head and thorax. In the Brachyura
the homologous suture is usually sought in the depressions
surrounding the cardiac region of the carapax. The true
explanations of these various structures was pointed out by
Dana ('52, pp. 23—28) over thirty years ago, and their total
neglect by all subsequent students is a partial excuse for their
mention here. The carapax of the lobster is wholly composed

[1] The peculiar lateral eyes of Euphausia are not included.

[2] Dr. Packard, in his text ('72, p. 165), speaks of the compound eyes
appearing " on the third segment of the cephalothorax," but his figures
(pl. v, figs. 24, 24a) show them in their proper position.

of the coalesced terga of the second antennal and the mandibular segments, and the " cervical suture " indicates merely the line of their junction.   In the crab the homologous suture is to be sought on the deflexed surface of the carapax below the lateral margin.   The mandibular terga are comparatively small, and are the epimera of H. Milne Edwards.[1]   It is needless to say that I do not accept the opinions of Young ('80) on this subject, nor his views of the supra-œsophageal ganglia.

This same view is perfectly applicable to the Arachnida, where otherwise we should have to regard the eyes as distributed on different segments in different species and even in the same species.   A further confirmation is found in the fact that in the Scorpions (nor so far as I am aware in any Arachnid) the cephalothoracic tergum does not segment, or show any signs of the metamerism so evident on the ventral surface.   This point seems to me an additional argument in favor of the union of Limulus with the Scorpions.

The post-oral nature of the first pair of appendages and the non-appendicular nature of the metastoma needed to render valid the comparisons of Professor Lankester have been previously suspected or proved, and my observations are only confirmative, though in the case of the metastoma conclusive proof was hitherto lacking.

Unfortunately Metschnikoff's account of the development of the mesoblast is very scanty owing to the fact that he did not employ sections, and so I can only refer to Balfour's account of the Arachnida.   The process in Limulus and Agalena is closely similar and in its details considerably different from that found in Crustacea.   In the latter group the mesoblast does not as a rule become divided into somites,[2] nor does the schizocœle extend into the legs at first.   In both Agalena and Limulus it arises as a single broad sheet, which later divides into two bands which migrate to the region of the appendages.   Then the cœlom is formed by splitting and

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[1] ' Hist. Nat. Crust.,' pl. i, fig. 9, b.

[2] It does so in Mysis (Metschnikoff, teste Balfour) and in Cyclops (Urbanowicz in '84).

extends into the legs. The mesoblastic processes in both divide the yolk up into a series of lobes, but these partitions never reach clear across the body. A similarity is observable in the late appearance of the lumen of the alimentary tract *cf.* Isopods), and apparently in both (certainly in Limulus) the hepatic organs are formed from the lobes of yolk which extend between the mesoblastic partitions.

The yolk in Limulus never communicates with the cœlom as it does in many Hexapods and Crustaceans. Apparently the same is true with spiders.

The heart in Limulus arises by the hollowing out of a solid rod of mesoblast, its cells becoming transformed into blood-corpuscles. This is paralleled in spiders and in some Crustacea. Metschnikoff's account of the origin of the dorsal vessel in the scorpions is very improbable and needs confirmation before it can be accepted.

The close resemblances in the segmental organs of the adult scorpion and of Limulus have been pointed out by Lankester ('82 and '84), though in his later paper he does not refer to their possible homology with the segmental organs of worms, (suggested by Packard '75ᵃ) as he was unable to find any ducts. As described above I have found these ducts in the young, and doubtless the same result will be obtained when the young of the scorpion is studied by means of sections. This is the more probable since Bertkau ('84) has found homologous glands in various spiders, which were without external openings in the adult, but in the young of Atypus the duct was found to open at the base of the third pair of legs (fifth pair of appendages.)[1] Michael ('83, p. 21) describes similar glands in the Oribatidæ but failed to find their openings. The exact correspondence of these glands both in position and in their ducts, and their closely similar histological structure, is a point of no little importance in the argument for the union

[1] These glands have long been known in spiders, but had been regarded generally as belonging to the digestive tract. They were practically discovered and their true structure first described both in Scorpions and Spiders by Professor Lankester.

of the Arachnids and Limulus in a common group.   It should be noted, however, that in complication, size, &c., the resemblance between the glands of Limulus and those of the spiders is closer than between those of Limulus and those of the most limuloid of Arachnids, the scorpions.

Packard ('75ª), discovered these organs and suggested their homology with the segmental organs of worms; Lankester ('82, p. 101) said "Possibly such coxal glands are the modified and isolated representatives of the complete series of tubular glands (nephridia) found at the base of each leg in the archaic Arthropod Peripatus;" while Michael ('83) suggests the homology of the glands in the Oribatidæ with those of Scorpio, Limulus, Crustacea and Worms.   Packard, in a later paper ('83), while failing to see the argument for the close association of the Arachnids and Limulus derived from this organ, recognises the correspondence between the glands of Limulus and those of the Crustacea; but instead of making his comparisons with the "shell-gland" he refers only to the "green-gland" of the Decapods, an organ which occupies a different position in the body.   His conclusion that "The occurrence of these organs in the Arachnida, as well as in Crustacea, indicates the independent origin of these two groups of Arthropoda" is intelligible only on the supposition that the word here italicised is a slip of the pen.   This similarity of segmental organs in Limulus and the Arachnida neither loses nor gains in force by a comparison with the shell-gland of the lower Crustacea.   This organ is a coiled tube with a cœcal inner extremity and an efferent duct which opens at the base of the second maxilla (a position which I shall show further on is exactly comparable to that where the segmental organs of Limulus and the spider's empty) but differs from the glands in the other groups in retaining its external opening through life.   Thus the coxal glands of the one are the exact homologous of the shell-glands of the other.

In the Crustacea two of the primitive series of segmental organs are found, one being the shell gland, the other the organ variously termed antennal gland or green gland.   Grobben

('80, p. 103) says that the antennal and shell glands agree in structure. Both terminate cœcally, and have a long convoluted duct opening at the base of the corresponding pairs of appendages.

The homology of the shell gland of the Crustacea with the segmental organs of the worms has been alluded to by many observers since Leydig first suggested it. Their similar origin,[1] structure, position, and ducts, all seem to point to their homology. The strongest arguments against it are that the series contains at the most but two pairs,[2] and that no connection with the body cavity exists. Recent authors (Leydig, '78, Weissman, '74, and Urbanowicz, '84) seem to strongly favor the homology.

The consideration of the evidence presented by the nervous, digestive, and circulatory systems I leave for another article; that by the respiratory system has already been alluded to. It may be well here to combine the various points of similarity between Limulus and the Spiders, on the one hand, and the Crustacea on the other. In this I embody the results gathered by Professor Lankester in his valuable paper ('81), so often referred to.

Limulus agrees with the Arachnida and differs from the Crustacea (1) in having six pairs of primitively post-oral pediform appendages; (2) a seventh pair bearing the outlets of the reproductive organs; (3) the ninth, tenth, eleventh, and twelfth, modified for respiratory purposes (the eighth pair in each shows a readily homologised structure, but different functions) ; (4) in the character and structure of the upper lip; (5) in the presence of a metastoma derived from the sternal portion of the sixth cephalothoracic segment.

[1] Reichenbach ('77) claimed that in Astacus the antennal gland was epiblastic in origin, a mistake which was corrected by Grobben ('79). Though Ishickawa ('85) repeats the statement for Atyephira.

[2] Huet ('82, I have not seen his final paper) describes a series of what he regards as segmental organs in the thorax of terrestrial Isopods (Oniscidæ), which are arranged one pair in each segment. The caudal glands of Lereboullet ('52) would appear to belong to the same series. Some of these have been found in the aquatic Oniscidæ.

The same agreements and differences are seen in (6) the possession of an entostermite with the same shape, position, and histological structure ; (7) in the backward extension of the tergum of the head, so as to form the dorsum of the cephalothorax ; (8) in the distribution of the eyes ; (9) in the existence of a blood colored blue by the presence of hæmocyanin (10) in the branching and anastomosing spermatic duct (Benham, '83) ; (11) in the mode of formation of the mesoblast, its segmentation, and the early extension of the body cavity into the limbs ; (12) in the mode of formation and structure of the alimentary canal and its appendages ;[1] (13) the elongate stomodæum and the short proctodæum ; (14) in the origin of the sternal artery and its intimate association with the ventral nervous cord ; (15) the early closure of the duct of fifth (the only) segmental organ ; (16) the absence of the second segmental organ (antennal gland).

Besides these points there are a number of other points in which the two agree, and which are but rarely paralleled in the Crustacea, and then not in groups which anyone would think of placing near Limulus.   Among these may be mentioned the possession of vibratile spermatozoa (paralleled in the barnacles[2]), the concentration of the nervous system around the œsophagus (also in Brachyura), a brain which never supplies any of the appendages.

This array of similarities is almost conclusive, but we must turn to the other side of the question, and seek the arguments against the association of Limulus with the Scorpions.   Dr. Packard is by far the most able advocate of the Crustacean affinities of Limulus, and in his latest paper ('82), in which he deals with this subject (confessedly a reply to Lankester's "Limulus an Arachnid"), we find the following summary of the arguments against placing Limulus in the Arachnidan phylum.   In the quotation I have inserted numerals for convenience of reference below : "At the outset it will be remem-

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[1] These points will be discussed in the second part of this paper now in preparation.

[2] They also have a slight motion in some other Crustacea.

bered that Limulus differs from the Tracheates, including the
Arachnids, (1) in having no tracheæ, (2) no spiracles, and (3)
no Malpighian tubes. It differs from the Arachnids in these
characters, also (4) in having compound eyes, (5) no functional
mandibles or maxillæ, (6) the legs not terminating, as generally
the case in Tracheates, in a pair of minute claws, while (7) its
brain does not, as in the Arachnida, supply both eyes and the
first cephalic appendages. On the other hand, Limulus agrees
with the Crustacea (8) in being aquatic (9) and breathing by
external gills attached to several pairs of biramous feet, in
having (10) a simple brain, which, as in some typical Crus-
tacea (Branchiopoda, &c.), does not supply any of the appen-
dages, while the structure of (11) the circulatory, (12) inges-
tive, and (13) reproductive organs agrees with that of the
Crustacea; and, (14) as we have shown in our embryology of
Limulus, ... the development of Limulus is like that of
certain other Crustacea with a condensed metamorphosis, (15)
the possession of an amnion being paralleled by that of Apus.
In all essential points Limulus is a Crustacean, with some fun-
damental features in which it departs from the normal Crusta-
cean type, and with some superficial characters in which it
resembles the Scorpion."

Of these points numbers 1, 2, and 15 have already been dis-
cussed in this paper, while numbers 5, 6, and 8 are trivial and
of no importance. Since Dr. Packard wrote the above, Mr.
Benham ('83) has shown that the thirteenth of these points is
not true, while the answer to 11 may be found in Professor
Lankester's paper. In regard to number 12 I would say that
in development, except to a slight extent in some Tetradeca-
pods, Limulus does not agree with the Crustacea, the hypoblast
being solid and a midgut not appearing until after hatching.
The origin of the liver and the structure of its ducts are
greatly different in the two groups. Points 7 and 10 are the
same and deserve more attention. It has been shown by
Balfour and by Schimkewitch that in the spiders the ganglia of
the first pair of appendages are primitively post-oral, and that
with development they acquire a pre-oral position and eventu-

ally fuse with the "brain." In Limulus the same process occurs but stops just short of the fusion of the corresponding ganglia with the pre-oral ones. For a knowledge of the condition of the brain in the Scorpions, one must await a detailed account of the structure. As the case is at present Dr. Packard distinctly states ('80ᵃ) that the brain supplies the first pair of appendages ; while Newport's figure ('43, pl. xii, fig. 15) shows the nerves as arising from the side of the brain. Newport was a very careful worker but the subject needs further study. On the other hand, in all the Crustacea, not excepting Apus and Limnetis (Branchiopoda), this coalescence of ganglia has gone still further than in Limulus or Arachnids, and even in the earliest stages the first pair of appendages are pre-oral in innervation, while the ganglia of the second pair (Apus excepted) move forward with growth from a primitively post-oral position and form an important part of the brain.

Fourth in the series comes the presence of simple lateral eyes in the Arachnids and compound eyes in the other. I have not yet studied my sections of the eyes of the young carefully enough to throw any light on the question. Since Dr. Packard wrote, Lankester and Bourne have shown ('83) that if a comparison be made of the whole compound eye of Limulus with the entire lateral group of the Scorpion the correspondence is very nearly perfect; and that " if we supposed a common ancestor of the Scorpion and King Crab to have exhibited a lateral 'ocular area' which possessed a single feebly developed cuticular lens, then by two slightly divergent lines of differentiation we can obtain the grouped eyes of Scorpio on the one hand, and the polymeniscous eye of Limulus on the other hand." The same authors also show that " the essential agreement of the central eyes of Limulus with those of Scorpions is obvious." As to Professor Lankester's well-known accuracy in histological work no comment is necessary and no confirmation is needed, but I would say that before his results were published I carefully studied the eyes of the adult Limulus, and so far as that

animal is concerned I can vouch for the accuracy of his account.

In regard to the eighth point of Dr. Packard's paper, it will at once be recalled that the gills in the Crustacea are formed on several distinct plans, and an effort to homologise those for instance, of forms so closely related as the Lobster and Squilla is not easily carried out. I regard the gills of Limulus, like those of Apus, as derived from some ancestral form with expanded and flattened appendages, which exposed a large surface to the water and hence became largely the seat of respiration. With a thickening of the cuticle and increase in size, the necessity for increased respiratory surface led in Limulus to the formation of outgrowths (gill-lamellæ) from these appendages just as occurs (though not from the same reason) in the individual to-day. The not very plainly marked biramous character of the abdominal appendages of Limulus is an ancestral feature which may have been lost in the Arachnid stems from the early change which they undergo. Nothing approaching a biramous condition is found in the six cephalo-thoracic members of either spiders [1] or horseshoe crabs except in the sixth pair of the latter, and to homologise the joints of that member with the protopodite, exopodite, endopodite, and epipodite of the " typical " crustacean limb is a task that I do not care to undertake. It seems, on the other hand, to be much more like one of the thoracic feet of Apus.

The condensed metamorphosis mentioned in the fourteenth point is, I suppose, another method of saying that the development is direct, for certainly Limulus shows nothing that could be regarded in the light of a metamorphosis, and it is just this lack of any larval forms which renders it so difficult to decide upon the affinities of the forms in question. Had we any nauplius or zoea stage the problem would be an easy one to solve. As it is the development is direct just as it is in Tetra-decapods, Spiders, and many other forms.

The third of the points is the most difficult to explain. In

[1] Croneberg's observations on Dendryphantes ('80, pl. xvi, fig. 14—16) need confirmation.

both Hexapods and Arachnids two or more urinary tubules are developed from the proctodæum (their development in Arachnida has not been described); in Limulus nothing of the sort is found, nor is anything of the kind certainly known in the Crustacea. As I shall have again occasion to refer to these organs I will leave the enumeration of the horns of the dilemma until then.

The close relationship existing between Limulus and the Trilobites has often been insisted upon by naturalists since Lockwood first saw a young horseshoe escape from its egg. The recent work of Walcott ('81), though I cannot accept his interpretations in many respects, serves to show that the resemblance between the two are not so great, or rather the differences are too great, to warrant a close association of these forms. This is more apparent from the discovery of the Ohio specimen described and figured by the same gentleman ('84). The veteran carcinologist, Henri Milne Edwards ('81), also fails to recognise these affinities, though he places his objection on different grounds from those that I hold. I do not care to enter into a discussion of the problem, but would state that according to these results and specimens the Trilobites had a series of non-chelate ambulatory limbs extending to the extremity of the body. Each limb consisted of a basal joint, from which arose an endopodite with six cylindrical joints, a three-jointed setose epipodite (exopodite?), and outside of these, just as in the lobster, the branchial organs. These latter were filamentary and straight or spirally coiled. With such a different appendicular structure it seems to me that we must have more than a strained resemblance of dorsal surfaces before admitting any close resemblance between the two, though it must be said that there is a certain resemblance between the four anterior legs of the Trilobite and their relationship to the mouth (as restored by Walcott, '81, pl. vi, fig. 1) and the four posterior cephalothoracic limbs of Limulus.

If we are to accept these resemblances as indicative of a homology between the two we must conclude that the first two pairs of appendages of the Trilobites have been lost, to say

nothing of the change in form and function of the abdominal appendages. It also necessitates a change in the nomenclature of the regions of the Trilobite body. The head will correspond to the cephalothorax of Limulus, and the thorax and abdomen or pygidium together will equal the abdomen of the horseshoe crab.

The Systematic Position of the Arachnida.

Since 1858, when Leuckart divided the old group Articulata into Worms and Arthropods, and the latter group into the equivalents of Branchiates, not a single author, so far as I am aware, with the exception of Van Beneden and Lankester, has questioned the close association of the Arachnids with the Hexapods and Myriapods in a common group called either Insecta or Tracheata. Entirely independently of Professor Lankester's papers ('77 and '81) I came to somewhat similar conclusions, which may be stated as follows :—Omitting Peripatus, the Arthropods should be divided into three equivalent groups or classes, one embracing the Crustacea, the second Limulus and the Arachnids, and the third the Hexapods and possibly the Myriapods. The last of these for convenience may be called Insects, the second Acerata (a modification of the name given by Latreille to the Arachnida alone). The Crustacea and Acerata are more closely allied to each other than either to the Insects, and the nearest representatives to-day of their common ancestor are Limulus and Apus. Before these two classes diverged the Insects had left the primitive Arthropod stem.

The points of difference between the Arachnids and the Insects are many, those between the former group and the Crustaceans less in number and more readily to be considered as derivations of a common ancestor. These points and their bearings I will consider in a rapid manner beginning with the anterior appendages, the posterior in all of the groups showing too many variations to afford any decisive results.

First, in the Insects we have antennæ which are primitively pre-oral, and not to be homologised in position or character

with any structures as yet found in Crustacea or Acerata. It has been shown by several students that in the latter group all of the appendages are, in the embryo, post-oral both in position and in nerve supply. The same is true of the second pair in the Crustacea, and in the adult Phyllopods (which are admitted by all to be the most primitive of the Crustaceans), both anterior pairs receive their nerves from the œsophageal commissure, the corresponding (?) ganglia being distinctly post-oral in position.[1] It would appear possible that in the very early stages of other Crustacea the same condition may exist, as in several forms the rudimentary first pair of appendages has not a distinctly pre-stomial position. The first to suggest itself is Moina, where, according to Grobben ('**79**), the first pair of appendages to appear have a position decidedly posterior to the stomodeal invagination. This pair Grobben interprets (and possibly correctly) as the second antennæ, but there is not certainty on this point. In the Nauplius of Palæmon, according to Bobretzky[2] (**73**), the first pair are on a level with the labrum, and show distinctly the similarity to the rest of the post-oral series. In the corresponding or a little earlier stage of Astacus (Reichenbach, '**77**, pl. x, fig. 8) they are some distance behind the labrum and the oral depression. The same is true of Eupagurus (Mayer, '**77**, pl. xiii, fig. 18) and of Crangon (my own studies).

The antennæ of Insects, on the other hand, always arise from the procephalic lobes. The possibility that Peripatus is a but slightly modified descendant of the ancestors of the Hexapods and Myriapods makes its evidence of some importance in this connection. Balfour, after a careful study of the anatomy, concludes that in Peripatus ('**83**, p. 217) "the antennæ are prolongations of the dorso-lateral parts of the anterior end of the body;" and his figures show that the eyes intervene between the antennæ and the other appendages. Kennel, from a study of two other species of the same genus,

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[1] Pelseneer's results as to the brain of Apus ['**85**] require a modification of this statement.

[2] Teste Faxon ('**82**, pl. xi, fig. 6).

comes to a similar conclusion, and carries it further, as follows
('**84**, p. 200):—" Obwohl nun dieses segment [the ' praeorale
Abschnitt'] seiner Entstehung nach allen anderen des Korpers
homodynam ist, glaub ich doch es denselben gegenüberstellen
zu müssen da es sich in ganz anderer Weise umbildet und
niemals Organe erzeugt, wie sie allen anderen Segmenten aus-
nahmlos eigen sind ; es entstehen in ihm keine Segmental-
organe, keine Drüsenbildung, auch keine Extremitäten ; denn
ob die Tentakel für Gebilde gehalten werden dürfen, welche
den Extremitäten der Rumpfsegmente homolog sind, ist auch
bei den übrigen Tracheaten, sofern man die Antennen derselben
als gleichwertige Bildungen durch die ganze Reihe hindurch
auffast, nicht ausgemacht."

A considerable difficulty arises when we try to homologise
the antennæ of Insects with those of Peripatus.   In the latter,
as has just been said, these organs receive their nervous supply
from the brain in front of the eyes.   In Myriapods, according
to Newport, the case is the same ; but in Hexapods the case
seems to be different ; but careful study is yet needed to settle
this point.   In the embryo Hexapod the antennæ rise from the
posterior side of the procephalic lobes, and there appears to be
much evidence that they are innervated from a distinct ganglion
from that which supplies the eyes.   On the other side, Ayres
('**84** pl. 20, figs. 22 and 23) represents the antennal lobe in
Œcanthus as being in front of the ocular lobe and between it
and the origin of the nerves to the ocelli, a condition which
needs confirmation.

Should this view that the antennæ of Peripatus and Hexapods
are not homologous prove true it would throw considerable
doubts upon the comparatively close relationship which has
been supposed that they hold to each other, a relationship
which may be doubted on several other grounds, some of which
will be mentioned later.

In the Spiders but one recent author (Croneberg, '**80**) has,
so far as I am aware, found what he regards as antennæ.   He
figures (pl. xvi, figs. 14—16) the embryo of Dendryphantes,
showing the upper lip (rostrum) arising as two appendage-like

lobes, in front of the rudimentary cheliceræ and between the procephalic lobes.   His figures clearly show however, that on account of their relations to the nervous system these lobes cannot be regarded as homodynamous either with the post-oral appendages, or with the appendages of insects.

Assuming for the moment that the view that the antennæ of Insects are not represented in either the Acerata or the Crustacea is correct, a comparison of the three groups may give us some further interesting points.   In the following schedule only the anterior segments are included, and the appendages are called by their usual names, which no one regards as indicative of homologies extending through the whole Arthropodan phylum.

Hexapoda.		Acerata.		Crustacea.
(1) Antennæ	. .	Absent	. .	Absent.
(2) Mandibles	. .	Cheliceræ .	.	Antennulæ.
(3) Maxillæ	. .	Pedipalpi .	.	Antennæ.
(4) Labium	. .	1st legs .	.	Mandibles.
(5) 1st pair legs .	.	2nd pair legs	.	1st maxillæ.
(6) 2nd pair legs .	.	3rd pair legs	.	2nd maxillæ.
(7) 3rd pair legs .	.	4th pair legs	.	1st maxillipeds.

This comparison is a strictly serial one and starts on the assumption that the first pairs of primitively post-oral appendages are homologous throughout.   That this is true in the cases of the two last groups is, to my mind, very probable, but with regard to the Hexapods there is some reason for doubt. In following it out we are led to some interesting regional coincidences.   It brings the end of the thorax of the Hexapod and Spiders into exact correspondence.   In the case of the Crustacea a line of demarcation at the same spot is frequently visible, and one needs but to mention that it corresponds exactly to the posterior end of the head of the Tetradecapods, and is well paralleled in Squilla, and in the larva of Palinurus. In the Entomostraca a division at the same point may be seen in the larvæ as also in the protozoea of Lucifer.   These may all be analogies, and the fact that no such regional distinctions

are found in the most primitive group of Crustacea, the Phyllo-
pods, is against their having much significance.

The comparison also leads to another correspondence in the
Crustacea and Acerata, which goes as far to prove its validity
as did the regional comparison in the case of the Spiders and
Hexapods. In both Crustacea and Arachnids the segmental
organs (shell glands of the former, coxal glands in the latter)
empty at the same point; at the base of the third pair of
walking legs in the Spiders and Limulus, and at the base of the
second maxillæ in the Crustacea, or, in other words, at the base
of the fifth pair of appendages in each. It seems to me that
this persistence of exactly the same segmental duct in these
two groups and the absence of any corresponding organs in the
Hexapods is an argument of no little weight.

If, however, it be shown that a variation or a disappearance
of segments or appendages may take place at the anterior end
of the Arthropod body this comparison will lose part of its
force. That such obsolescence in the adult occasionally occurs
is well known, but the presence of the appendages in the
young readily enable us to check our results. Setting aside
the parasitic forms the best known cases are presented by
certain Copepods, Apus, and the Oniscidæ, but the fact that
the normal condition is found in their near relations shows
that this fact is of no morphological importance in this con-
nection. In Eurypterus, according to Lankester, there is a
case of an apparent disappearance of one pair; but until it is
proved that the Trilobites are related to the Acerata (a point
on which, as mentioned above, Walcott's observations throw
considerable doubt) the assumption that the cephalic buckler
of these forms is the exact homologue of the cephalothorax of
Limulus is not warranted, and in case it turns out that the
two are different these fossils throw no light on the subject.
Until it is proven that a segment or an appendage may dis-
appear in the anterior end of the adults of a large group of
animals, it is justifiable to assume the exact homology of the
first pair in all three groups, as I have done above. It is
possible that an embryological study of the cement glands,

together with the mandibular glands of Hexapods and the cheliceral glands of Spiders, may throw some light on this point.

If, as pointed out by Lankester, the tracheæ of the Arachnids have arisen from the gills of the Limulus,[1] then either those of the Hexapods must have been derived from those of the Arachnids, or those in the two groups must have arisen independently. The latter I believe to be the true case, and without entering into any argument to establish this point, I would call attention to the fact that in the terrestrial Isopods (Oniscidæ) the gills become permeated with trachea-like air-tubes. Long ago Lereboullet ('52, pl. vii, figs. 148 and 149) figured these ramifying tubes, while, to consult a more recent student, Leydig ('78, p. 265, pl. xi, fig. 32) describes the mode of their formation. The cells of the blood-spaces of the gills secrete an internal cuticula, and in the branching cavities which this contains the air circulates. This forms tracheæ, certainly without any phylogenetic connection with those of the so-called tracheates, but which present many analogies with them. It also affords good grounds for the supposition of Professor Lankester that the trachea, at least in some groups, have followed the course of pre-existent blood-vessels.

Another fact of some weight in this connection lies in the position of the tracheal openings. In the Arachnids they

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[1] Schimkewitsch (**84**[a]) denies this, but offers no reasons, but rather seems to misapprehend the whole argument relative to the relationships of Limulus to the Arachnids. He says (l. c., p. 67), "Les poumons mêmes peuvent être considérés comme une modification des trachées en faisceaux des chenilles et des myriapods. Il est très probable que les ancêtres des Arachnides et des autres Trachéates avaient cette forme des trachées en faisceaux, laquelle a été transformée chez les Araignées en poumons. Tout cela me fait croire que les formes trachéennes et les Tetrapneumones sont plus anciennes que les Dipneumones." Further on (p. 84) he says, "Par leur apparail circulatoire et leur système musculaire, les Arachnides supérieures se rapprochent au contraire des Limulides ; mais cette resemblance peut être expliquée par l'identité qu'existe dans la configuration générale du corps de ces deux formes ; car les Limules, d'après leur évolution (état Nauplius et état de Trilobite) et d'après la constitution des l'appariel respiratoire, sont des vrais Crustacés privés d'antennes."

perforate the ventral plates, as also in the diplopod Myriapods. In the Hexapods and the chilopodous Myriapods the stigmata are placed outside these plates. These differences in position of the external openings may indicate a separate origin in all these groups, especially when we consider Sedgwick's specula- tions ('84) on the origin of tracheæ. Again, it should be noticed that no traces of cephalothoracic stigmata or tracheæ occur in the Spiders.

One objection to the separation of the Hexapods and Arach- nids, and the closer connection of the latter with the Crustacea, lies in the fact that biramose appendages are characteristic of the branchiate and simple of the tracheate Arthropods. This, however, may be regarded in two lights. In the an- cestral Arthropod both exopodite and endopodite may have been present, and both branches may have been retained in the aquatic and only one in the terrestrial forms, or the ancestor may have had broad and flattened but unbranched appendages which have been variously differentiated in different groups. There are several facts in favor of either of these views, though the evidence presented by Apus, together with many other points render the latter the more probable. To mention one or two of these: Apus is by nearly all morphologists regarded as the most ancestral type of Crustacea. Its members (Lankester, '81[a]) are broad and flattened plates bearing typically six internal and two external lobes, but studying the adult alone no one would ever arive at the idea of exopodite, endopodite, and epipodite. In the Nauplius stage, in the second and third appendages, a biramose condition exists. This is, however, to be regarded as secondary in its nature, just as is the Nauplius itself. In the maxillæ of forms even as high as Decapods, this lamellar condition persists, and all attempts to trace the homologies of exopodite and endo- podite meet with but partial success.

The evidence on the other side is fragmentary. Croneberg ('80, pl. xvi, figs. 14—16) describes and figures an embryo Arachnid (Dendryphantes) in which the pedipalpi are distinctly bifid at the tip. This, however, has never, so far as I am

aware, been seen by any other observer, and needs confirmation. Among other groups of Tracheates are the peculiar antennæ of the Pauropids, with their basal joints bearing two branches, one of which in turn is bifid. The investigations of Mr. Wood-Mason (’79) show that in some of the Thysanura and in the Cock· roaches some of the appendages exhibit a biramose condition. While some of his facts and many of his speculations need confirmation,[1] I think that in some instances (enough for our purposes) he has proved his points. Probably more extended studies on the Thysanura will throw important light on the relationships of the Hexapods to other forms. Patton (’84, p. 596) describes a similar condition in Blatta germanica: "A rather striking variation was found in the first and second maxillæ of Blatta, which were formed respectively of two and three branches, the second maxillæ thus attaining the typical trichotomous structure of the Crustacean appendages."

The existence of Malpighian tubes in Spiders and Hexapods is, without doubt, the greatest obstacle to the arrangement of the Arthropods here advocated, since they are absent in Limulus and the typical Crustacea. Their mode of development debars us from considering them as modified segmental organs, and we can only say that they are either inherited from an ancestor common to all Arthropods and have disappeared from some groups, or we must admit that they have appeared independently in two or more branches of the Arthropods. Which of these two alternatives we must take cannot be at present decided, though some facts may throw light upon the subject. The first thing that we notice is that those groups (Hexapods, Myriapods, and Arachnids) which possess them are terrestrial, while the great bulk of the Crustacea are aquatic ; and this at once suggests that the organs may have an origin from similar physiological causes without any phylogenetic connection existing between them. Turning to those Crustacea which lead a terrestrial life we meet with some striking confirmations of this hypothesis.

Zenker (’54, pp. 106, 107) describes in the young Asellus

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[1] *E.g.*, The structure of the mandible in Machilis.

(a fresh-water form) six glandular spots on either side of the abdomen, which later unite to form a tube, though he did not find the place where it opened. Weber ('79, p. 237) mentions and ('81, pp. 608—612) describes and figures tubes emptying into the hinder intestine in Trichoniscus, and says that they also occur in other Oniscidæ. He also states that he found depositions of urates, a fact which at once deprives the opinion of Wrzesniowski ('79, pp. 514, 515) of much of its force. The investigations of Nebeski ('80, pp. 122—127) are the most interesting. This author describes certain organs arising at the beginning of the hind gut of certain Amphipods. In the strictly aquatic genera these glands are small, but in the more terrestrial Gammarus they become well developed, while in Orchestia, a form living in the sand above high tide, they are very long and tubular, and the histology shows that they have active secretory or excretory functions. In position and origin they correspond closely with the Malpighian tubes. His series on pl. ii, fig. 14, is very instructive.

Nebeski's observations, however, are not conclusive, for he shows the rudiments of the same organs in Amphipodous genera which are solely aquatic. Gamroth ('78, pl. x, fig. 14) figures two globular glands occupying the same position in Caprella which he regards as urinary, and Haller ('80, p. 384), studying the same genus, agrees with him. These forms are all confined to the Tetradecapods, and so far as I am aware, no similar organs have been found in any other Crustacea. Still they may be derived from an ancestor common to them and to all the Tracheates. These ancestral glands may have been very small, possibly mere glandular surfaces, and changed conditions in life have caused their increased development. A somewhat parallel case is that noted by Grobben ('80) that the antennal gland differs in its length and complication in the salt- and in the fresh-water members of the same genera of Copepoda.

This of course is but analogy. It seems at present as if these organs had arisen in the Crustacea independently of their existence in other Arthropods. If this be the case, their

existence in Hexapods and Myriapods loses some of its force. If the reverse be true the argument, derived from these organs, for the maintenance of the group Tracheata has even less weight.

From these and other facts it seems to me probable that the ancestral Hexapod left the main Arthropod stem some time before the separation of the Crustacea and Acerata. The common ancestor of all three was an elongate animal with flattened ambulatory appendages, some of which (except possibly the first) were adapted for the purposes of eating. The head bore on its dorsal surface scattered sensory (optic) organs. In most, if not all, the segments of the body were segmental organs, through which passed the products of the genital glands, no genital ducts being differentiated. The reproductive glands were probably ventral in position; the nervous system consisted of a single supra-œsophageal ganglion, a ventral chain and a supra-intestinal loop (*vide* Peripatus). The alimentary canal traversed the whole length of the body; and, contrary to the opinions of the Hertwigs ('**81**), it seems probable that circulation was effected by the pulsation of the dorsal splanchnopleural muscles, the cœlom containing the circulatory fluid, and that this portion was constricted off to form the heart.

The Hexapods left this stem at a time when the first pair of originally post-oral appendages were moving towards a pre-oral position.[1] The Spiders and Crustacea continued together until they had the following characters in common :—One pair of appendages had a distinctly pre-oral position, and the basal joints of at least the two succeeding pairs were employed in the comminution of food. The segmental organs had disappeared from the first, third, fourth, and sixth body segments and possibly from others. The genital glands became concentrated near the middle of the body, and the reproductive products passed out through the segmental organs in the same region. The genital glands themselves had assumed a dorsal

[1] Hatschek ('**77**) says that the ganglia of the mandible of the embryo Bombyx becomes transformed into part of the œsophageal connectives.

position. Most of the post-oral parts were concerned in respiration.

In the foregoing I have taken no account of Pauropus and the Myriapods, the facts concerning them being not yet well enough known. It seems possible that they have no connection with the Hexapods. The structure and relationship of the trachea, the ventral position of the genital glands, the mouth parts, the innervation of the antennæ, the existence of segmental organs in Peripatus and their possible homologues in the foramina repugnatoria of the Myriapods, all need to be taken into consideration in this connection.

I do not intend to defend in extenso these points at the present time; a few explanatory notes may, however, be of value. The relationship of the oviducts to the exterior in the Arthropods seems to point to the conclusion that here, as in many other animals, they are modified segmental organs. The positions in which these ducts empty would then warrant the conclusions given above regarding the relationships of the segmental organs. Several authorities (especially Grobben, '79 and '81[b]) have pointed out that the genital glands of Arthropods are ventral in origin, and that they later assume a dorsal position. This author also points out, as an indication of inferiority, that they permanently retain a ventral position in Peripatus and Myriapods.

Whether the first pair of appendages in the ancestral Arthropod played a part in eating is uncertain, though the facts that it now does so in the Hexapods, Myriapods, and Peripatus, and that in the other groups it at first has a post-oral position is in its favor. In Limulus, as in Apus, and in the Nauplii of various Crustacea, the basal joints of at least two pairs behind the first are so employed, and in the two forms first mentioned the series is extended further. In all it is the basal joint which is so employed, and in the young of some and the adults of others the distal portion is used for ambulatory purposes.

Note.—Since this article was sent to the printer several papers have appeared which have more or less bearing on the subjects here discussed, though

none cause any serious modification of the views here held. Professor Lankester ['85], entirely independently, has arrived at essentially similar views as to the homology existing between the lungs of Arachnids and the gills of Limulus. The causes assigned for the change differ, a matter of no importance in this connection. Mr. Pelseneer ['85] has shown that the brain of Apus is a "syncerebrum," a fact which modifies some of the statements in the text. The discovery by Mr. Spencer that the urinary tubules of the Amphipods are mesenteric ['85] is more important, though I must say that my argument demands analogies rather than exact homologies.

Dr. Packard ['85] has studied some sections of a stage about equal to my figure 5, and from them he draws the conclusion that the development of the brain of Limulus is entirely different from that of Arachnids. He distinctly states that the stage cut had the first pair of appendages in a post-oral position, but still he regards the nervous invagination in sections which pass through the first pair of limbs as the brain. This being the case, his argument falls to the ground. Contrary to his statements, I would say that the development of the brain of Limulus is closely like that of the Arachnids, and that Crustaceans do have procephalic lobes.

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EXPLANATION OF PLATES XXXVII, XXXVIII, AND XXXIX,

Illustrating Dr. J. S. Kingsley's Paper on " The Embryology of Limulus polyphemus."

Reference Letters.

a. Anus. *ab.* Abdomen. *ap.* Appendage. *b.* Modified blastopore. *bc.* Blood-corpuscle. *bl.* Blastoderm. *bs.* Blood-sinus. *c.* Cœlom. *cm.* Commissure. *cn.* Connective. *co.* Connective tissue. *ct.* Second larval cuticle. *cx.* Cephalothorax. *dt.* Deutovum. *e.* Eye. *ec.* Edge of carapax. *ei.* Epiblastic invagination for brain. *eo.* External opening of segmental organ *ep.* Epiblast. *f.* Flagellum of sixth pair of legs. *fn.* ? Funnel of segmental organ. *ga.* Gill-bearing appendage. *gl.* Gill-lamellæ. *gn.* Ganglion. *h.* Heart. *m.* Mesoblast. *mo.* Mouth. *mp.* Mesoblastic partitions. *mt.* Metastoma. *mu.* Muscle. *n.* Nerve. *nf.* Nerve-fibres. *ng.* Neural groove. *nr.* Neural thickening. *nu.* Nucleus. *o.* Operculum. *oc.* Ocelli. *p.* Proctodæum. *pab.* Abdominal appendage. *pc.* Procephalic lobes. *pl.* Pulmonary lamellæ. *pn.* Pulmonary invagination. *sg.* Segmental organ. *so.* Somatopleur. *sp.* Splanchnopleur. *st.* Stomodæum. *stg.* Stigma. *t.* Telson. *x.* Problematical organs of Fig. 3. *y.* Yolk. *yg.* Yolk-granules.

The Roman numerals, I, II, III, &c., refer to the segments; the Arabic, 1, 2, 3, &c., to the appendages of the body.

PLATE XXXVII.

FIG. 1.—Surface view of egg half an hour after attempted artificial impregnation.

FIG. 2.—Same egg twenty-one hours later.

FIG. 3.—Surface view of an egg twenty-nine hours after artificial impregnation, showing two peculiar protuberances on the surface. For detailed description of Figs. 1—3 see p. 544.

FIG. 4.—Early embryo of Limulus. The lighter area (*m*) indicates the extension of the mesoblast; the key-hole-shaped centre (*b*) is the modified blastopore. Osmic acid preparation.

FIG. 5.—Embryo after the appearance of the compound eyes (*e*), the six pairs of cephalothoracic appendages (1—6), and the operculum (*o*). The blastopore has now divided into the pyriform mouth and anus. The neural groove (*ng*) is seen. Osmic acid preparation.

Fig. 6.—"Germinal disc" of embryo after the appearance of the eighth (first gill-bearing) appendage (ga^1). The distinction between the cephalo-thoracic and abdominal regions is now distinct. Osmic acid preparation; camera drawing.

Figs. 7 and 8.—Surface and sectional views of deutovum after separation from the embryo. × 160.

Fig. 9.—Diagrammatic upper and, Fig. 10, side views of segmental organ, constructed from sections. I am not certain about the internal termination, *f*. Cf. Fig. 27.

Fig. 11.—Embryo at the time of moulting. The second larval cuticle (*ct*) viewed obliquely from the front to show the procephalic lobes (*pc*).

Fig. 12.—Embryo at the time it escapes from the chorion and assumes a Limuloid appearance. The position of the eye (*e*), extending across a meso-blastic partition, is noticeable.

Fig. 13.—Horizontal section of an embryo of the stage shown in Fig. 14, taken above the level of the eyes, to show the extent of ingrowth of the mesoblastic partitions (*mp*). × 14.

Fig. 14.—Under surface at the "trilobite stage," constructed from camera drawings. Owing to difficulties of manipulation the brain could not be seen, and the metastoma may not be correct.

Fig. 15.—Reproduction of Metschnikoff's figure ('71, Pl. xvi, fig. 12). "A part of the embryo [of Scorpio] to show the first formation of the lung." *p. ab.* Abdominal appendage. *pn.* Pulmonary sac.

Fig. 16.—Dorsal and, Fig. 17, ventral views of young Limulus at the escape from the deutovum and beginning of a free life. × 14.

Figs. 18, 19, and 20.—Diagrams illustrating the derivation of the pulmonary sacs of the Arachnids from the branchiæ of Limulus.

> Fig. 18. A single gill-appendage with the gill-lamellæ, as shown in the adult Limulus.
>
> Fig. 19. The gill-appendage is partially sunk in the ventral surface, and the gill-lamellæ exhibit a condition shown in Figs. 37 and 38.
>
> Fig. 20. Diagram of pulmonary sac of an Arachnid. The gill-lamellæ are replaced by the pulmonary lamellæ; the pit of invagination has narrowed above and forms a spiracle.

PLATE XXXVIII.

Fig. 21.—Transverse section through fourth segment of embryo, shown in Fig. 12. The mesoblast has met above and below. × 64.

Fig. 22.—Longitudinal section through the fourth to the seventh segments of an embryo in stage shown in Fig. 12. The section passes to one side of the median line, and shows the metastoma as a portion of the sternum of the sixth segment. × 76.

Fig. 23.—Longitudinal section of embryo, Fig. 12, showing the opening of the segmental organ and the vesicular enlargement. × 150.

Fig. 24.—Section through the nervous cord (not yet separated from the epiblast) behind the eighth ganglion of Fig. 12. × 380.

Fig. 25.—Section through seventh ganglion of Fig. 12. The separation of the nervous tissue from the epiblast is complete in the middle, though not at the sides.

Fig. 26.—Section parallel to and outside of Fig. 23, cutting through three folds of the segmental organ. A thirteenth of a millimetre intervenes between the two sections.

Fig. 27.—Transverse section of half of fifth segment, showing three portions of the segmental organ and its internal opening, *fn*. × 150.

Fig. 28.—Section of middle portion of segmental organ. × 1000.

Fig. 29.—Median sagittal section of embryo, Fig. 15. The section passes through the edge of the mouth (*mo*) and shows the epiblastic involution (*ei*) which later takes part in the formation of the brain. Though the first pair of appendages are distinctly preoral (cf. Fig. 12), the first ganglion of the ventral chain (*gn* I) retains a postoral position. Behind the eighth ganglion the nervous tissue has not separated from the epiblast. × 75.

PLATE XXXIX.

Fig. 30.—Transverse section of third appendage and adjoining region of Fig. 6 at the first appearance of the cœlom, showing it to be a schizocœle. × 150.

Figs. 31—33.—Formation of the heart, Fig. 31 being the most posterior. A twentieth of a millimetre behind Fig. 31 no lumen occurs in the solid mesoblast. A thirteenth of a millimetre intervenes between Figs. 31 and 32, and a fifth of a millimetre between Figs. 32 and 33. The conversion of mesoblast cells into blood-corpuscles is shown in all three figures. The thickening of the dorsal epiblast immediately above the heart is noticeable. × 442.

Fig. 34.—Constructed from three longitudinal sections of Fig. 6, showing portions of the cœlom in each segment of the body.

Fig. 35.—Transverse section through the middle of the fourth segment of Fig. 14, showing the segmental organs and the origin of the nerve to the fourth pair of appendages. × 75.

Fig. 36.—Section through the metastoma (*ep*) of Fig. 14, showing the mesoblastic outgrowths (*co*) to form the artery surrounding the ventral nervous cord. × 150.

Figs. 37 and 38.—Longitudinal section of abdominal appendages, Fig. 14, showing the mode of formation of the gill-lamellæ, the inpushing being as marked as the outgrowths. Compare with Fig. 19.

Fig. 39.—Origin of deutovum as a cuticle secreted by the blastoderm. The section is taken from an egg in the stage represented in Fig. 4. × 300.

Figs. 40—43.—Transverse sections of the mouth region of Fig. 6, illustrating the formation of the stomodeum by a closing-in of the epiblast. Fig. 40 is the most posterior. × 200.

Fig. 44.—Transverse section of the germinal area of Fig. 4, showing the origin of the mesoblast from the elongate "germinal groove" (modified blastopore).

Fig. 45.—Relative proportions of blastoderm and deutovum at the stage shown in Fig. 5.

Fig. 46.—Transverse section through anus of Fig. 5. × 175.

Fig. 47.—Transverse section through the fourth pair of appendages of Fig. 5, showing the extension of the mesoblast and the absence of a cœlom at this stage.

Fig. 2.
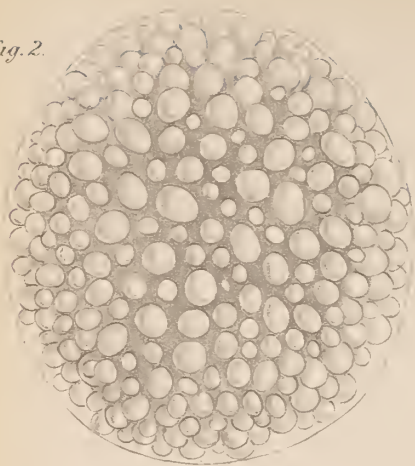
Fig. 3.

Fig. 4.

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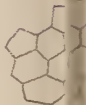
Fig. 8.

Fig. 12.

Fig. 13.

Fig. 16.

Fig. 17.

J S Kingsley del.
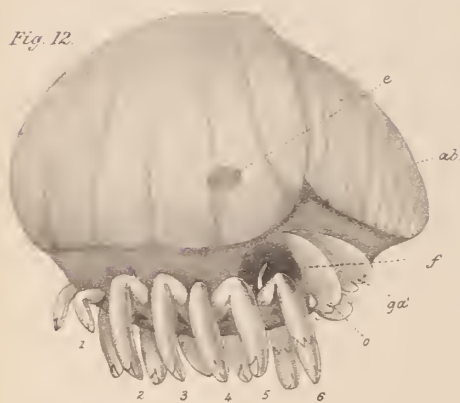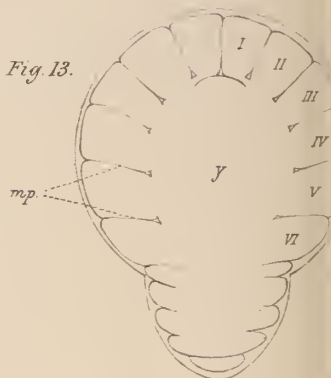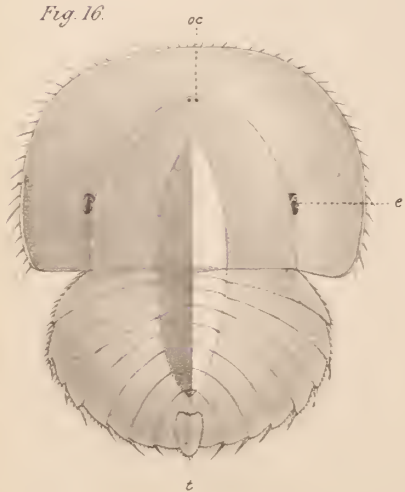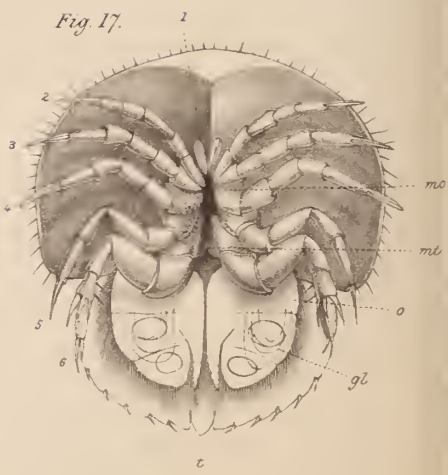
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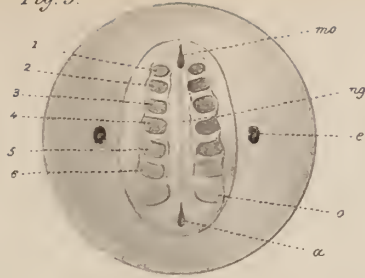
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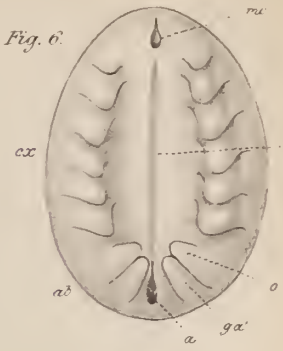
Fig. 9.

Fig. 10.

Fig. 11.
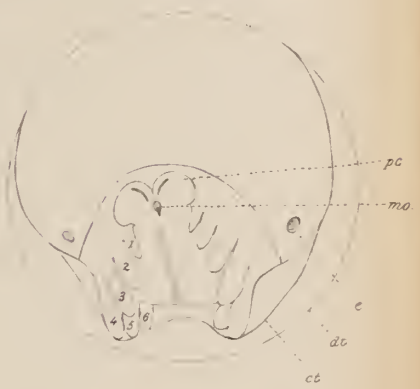
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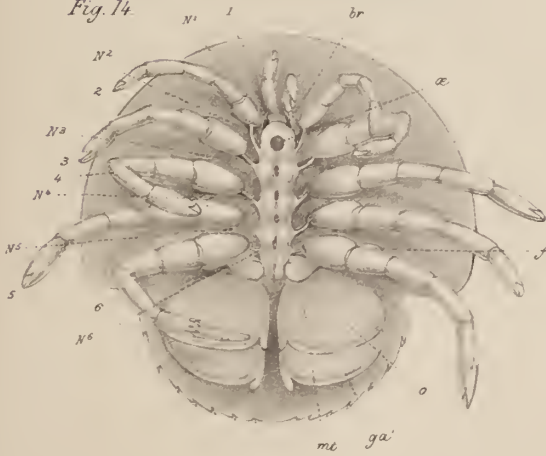
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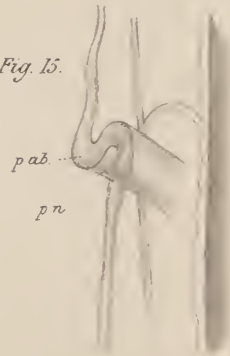
Fig. 18.

Fig. 19.

Fig. 20.

Fig 21

Fig 22

Fig 25

Fig 26

Fig 27

Fig 28
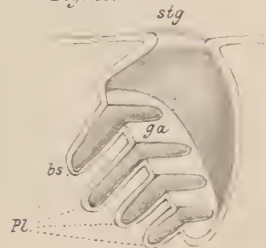
Fig 29

Fig 23
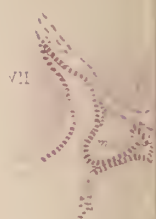
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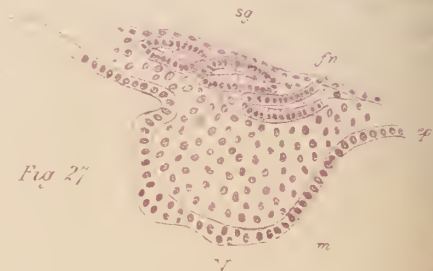
Fig 25

Fig 26.

Fig 27

Fig.29

Fig 28

Fig 23

Fig 24

gn.VIII gn.VII mt. gn.VI gn.V gn.IV gn.III gn.II gn.I
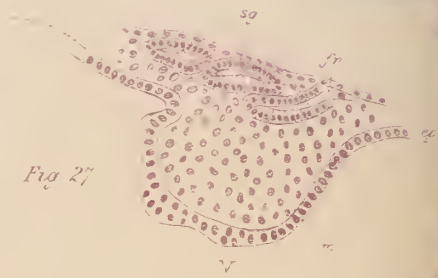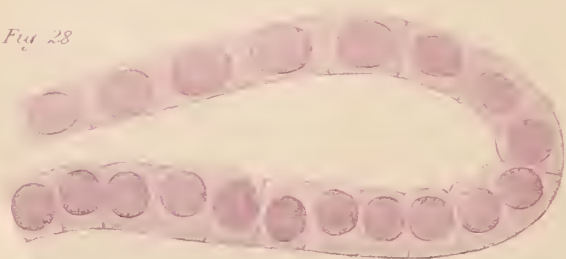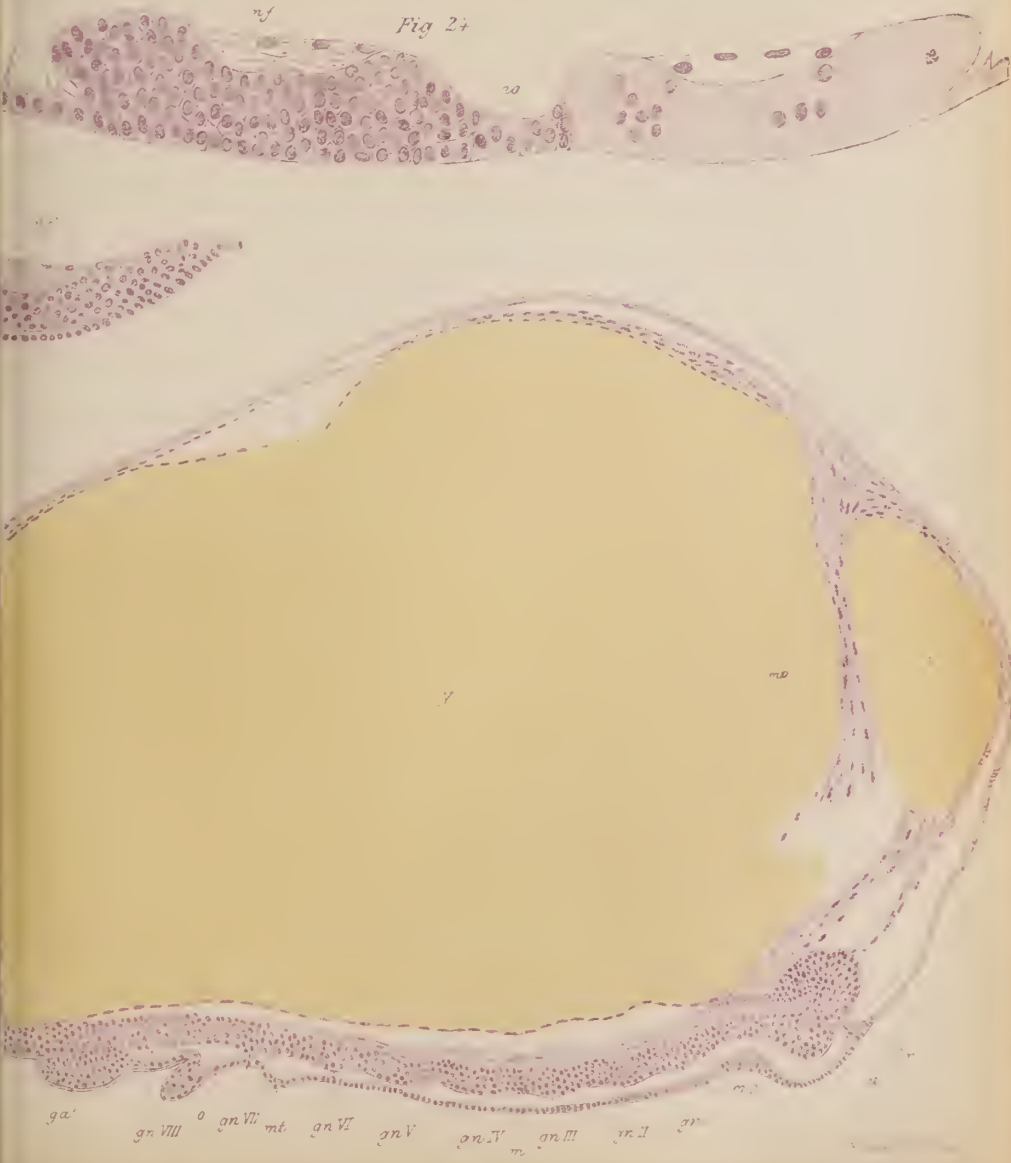
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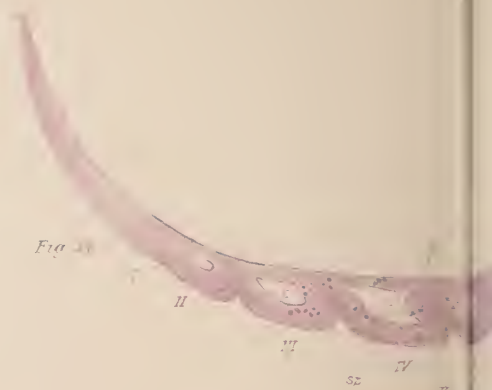
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Fig. 31
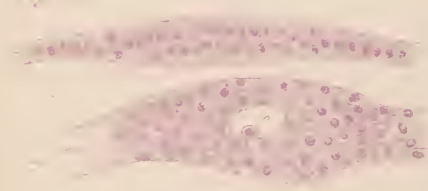
Fig. 35

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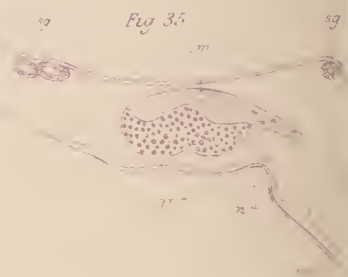
Fig. 36

Fig. 37

Fig. 33
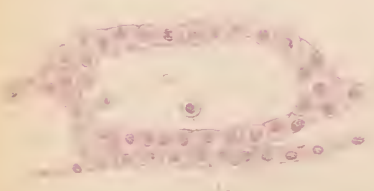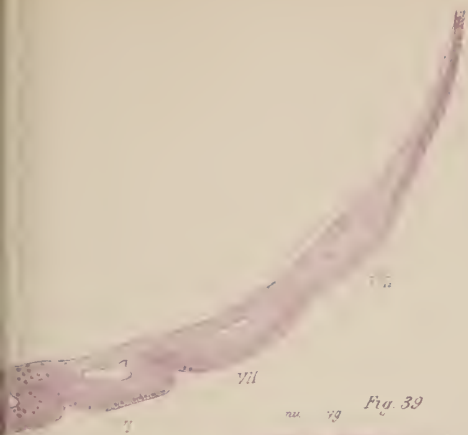
Fig. 40.

Fig. 41.

Fig. 42.

Fig. 39

Fig. 43
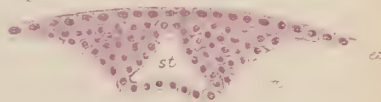
Fig. 38

Fig. 44
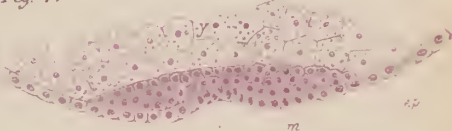
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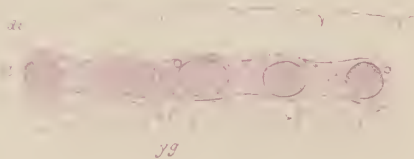
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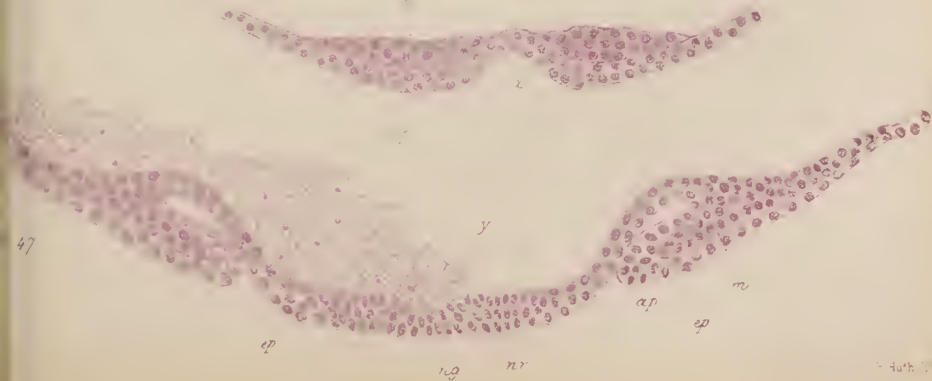
The Anatomy of the Madreporaria : I.

By

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Keble Coll., Oxon., Berkeley Fellow of the Owens College, Manchester.

With Plates XL, XLI, and XLII.

By the kindness of Professor H. N. Moseley I have been enabled to study the anatomy of certain Madreporaria obtained by him during the voyage of H.M.S. "Challenger." As in lecture-courses and text-books but little information is given relative to this ancient and interesting group, and the few papers on the subject are scattered, a short sketch of the more recent researches is prefixed to my own results. Two forms only are described in this paper, Flabellum patagonichum and Rhodopsammia parallela; others, it is hoped, will follow shortly. Throughout the text will be reduced as far as possible.

An acquaintance with the anatomy and development of an ordinary Actinia is presupposed in the reader, this being the type by which comparisons are made; but a list of the more technical names used to describe the anatomical parts of the polyp is given, with their synonyms for the use of those desirous of consulting the literature of the subject.

Mouth-disc = Mundscheibe, Peristome.

Body wall = Leibeswand.

Stomodæum (œsophagus) = Schlund-, oder Magen-rohr.

Cœlenteron = Darmhöhle, Leibeshöhle, Estomac.

Mesenteries (sarcosepta) = Scheidewände, Parietes, Replis mesenteroïdes.

Mesenterial filament (craspedon) = Mesenterialfaden, Cordon pelotonné.

Septa (sclerosepta) = Sternleisten, Cloisons, Lames.

Theca = Mauerblatt, Muraille.

A " pair " of mesenteries is constituted by two mesenteries whose longitudinal muscle-fibres are ranged on their adjacent faces, (except in the case of the two " directive pairs," each of which is placed at one end of the longer axis of the mouth oval, and in which the arrangement of the muscles is reversed). For the chambers (Radial-taschen, Loges,) into which the cœlenteron is periaxially divided by the mesenteries, I am compelled to coin new names; to those chambers which lie between a " pair " of mesenteries the term e n t o c œ l e is applied (fig. 1, B) ; to those chambers of which one lies between every two pairs of mesenteries the term e x o c œ l e (fig. 1, A). The septa lying in these two classes of chambers are similarly called e x o s e p t a and e n t o s e p t a.

The classification adopted will be found at the end of the paper, together with the bibliography.

RECENT RESEARCHES INTO THE MORPHOLOGY OF THE GROUP.

In 1873 Lacaze Duthiers (1), studying the development of **Astroides calycularis** on the coast of Algiers, found that it agreed in every important point with the development of Actinia, his observations on which (2) were corroborated and corrected by the Brothers Hertwig (3). With regard to the developing skeleton, he recorded two facts of importance: firstly, as appears in his pl. xiv, fig. 27, that it was formed o u t s i d e t h e p o l y p; and secondly, that the theca arose i n d e p e n d e n t l y o f t h e s e p t a. Owing to various practical difficulties his investigation was incomplete.

The chief worker in this field has been Georg von Koch,

who, in the course of several investigations, has arrived at the conclusion that the theca is a secondary structure, derived from fusion of the peripheral ends of the septa. The evidence adduced in support of this theory appears to me to be at present insufficient for complete proof, though from our slight knowledge of the group it is injudicious to absolutely deny its truth.

Von Koch first published this theory in 1879, founding it on the following observations on **Caryophyllia (4)**. There is no living tissue on the greater part of the exterior of the corallum, but at the apex the peripheral edge of the mouth-disc overlaps the lip of the calyx in such a way that in the highest sections the septa appeared to stand free in the cœlenteron, in sections a little lower to have fused peripherally into a theca. The costæ are, according to him, and as will be seen by the figures, the outermost ends of the septa (Pl. XL, A, B).

Further, the mesenteries and chambers between them appeared to be continued into this external part of the polyp. These appearances he explained by supposing that as the peripheral ends of the septa approximated and fused they surrounded the mesenteries, dividing them ultimately into a central and a peripheral part. As a further proof he adduced the observation that in microscopic sections of the corallum sutures were visible in the theca at the points where he supposed the septa to have fused.

I venture to think with Moseley (5) that this explanation is erroneous; that the appearances in the first section (Pl. XL, A) are due merely to the fact that in this, as in many corals, the secretion of calcium carbonate is most active about the septa, which consequently rise slightly above the level of the theca, as may be seen in any figure of Caryophyllia; and further that, in the second section (Pl. XL, B), the apparent continuation of the mesenteries and chambers between them over the tip of the calyx is not due to their having been cut into two portions by fusion of the septa, but to more or less abnormal contraction due to the use of alcohol; in life the polyp, when fully expanded, undoubtedly stretches over the

lip, but in these forms, so far as I can ascertain, in n a t u r a l
contraction it is completely within the calyx.   Further, as
appears from his own researches and those of others on
different forms, the whole skeleton, instead of being, as he
describes, free in the cœlenteron, is shut off from it by a layer
of endoderm and mesoderm, and as much outside it as the rest
of the corallum ; these layers he himself figures as clothing
another part of the septum, though of this portion no histo-
logical details are given.   Von Heider, in a paper shortly to
be referred to, states that von Koch has overlooked the fact
that the whole of the corallum is covered externally by ecto-
derm and mesoderm ; certainly this form requires more com-
plete investigation.   Again, having ground many microscopic
sections of corals I can afford no credence to "sutures ;" in
the process cracks fly through the coral in all directions.   But
if evidence of a directly contrary character is needed, the case
of Flabellum may be adduced, in which, according to Moseley
(5), sutures run, not between the fused ends of the septa, *i.e.*
through the theca, but down the centre of each septum.

Though von Koch gives no detailed description of the anatomy
of Caryophyllia, the following account may be inferred from his
figures and text (4) (6).   The polyp is built on the Actinian
type, consisting of mouth-disc, stomodæum, mesenteries ; the
muscles of the latter being arranged as in Actinia.   No external
body wall, its place being taken by the theca ; inner body wall
of mesoderm and endoderm, lining the cœlenteron, and clothing
the interior of the calyx, both theca, and septa.   Mouth-disc
drawn down in abnormal (?) alcoholic contraction over the
lip of the calyx.   Entosepta and exosepta both present.   No
mention made of tentacles.

Of **M a d r e p o r a   v a r i a b i l i s** he records, in 1880, the fol-
lowing facts (6).   Structure Actinian ; in the end-polyps of
the colony six pairs of mesenteries, six entosepta, and six
exosepta ; in the side-polyps also six pairs of mesenteries, but
six entosepta only.

V. Koch has also studied **S t y l o p h o r a   d i g i t a t a** in some-
what greater detail (7).   The form of the colony resembles

that of Alcyonium digitatum; the polyps live in small calyces
on the surface of the colony, but the living tissues are not
continued down into its centre, as in Alcyonium; the lower
part of the cavity formerly inhabited by the polyp being
shut off by a kind of tabula as it grows upwards. Over the
surface of the colony lies the cœnosarc, the fleshy rind of the
otherwise calcareous colony, which puts the polyps in commu-
nication with one another, being permeated by canals which
are continuous with their cœlentera, and similarly lined
by endoderm. The polyp possesses six pairs of mesenteries,
six larger tentacles, six smaller tentacles, and six entosepta.
There are two distinct types of nematocyst. Longitudinal
muscles occur on the mesenteries, but the smallness of the
latter rendered it impossible to detect whether their arrange-
ment agreed with Actinia or not.

In 1881, Dr. von Heider, of Graz, published a description
of Cladocora astræaria and Cl. cespitosa (8). These
species are also built on the Actinian type; and Heider
describes for them the same continuation of the mesenteries
and mesenterial spaces that v. Koch mentions as occurring in
Caryophyllia cyathus. I have examined macroscopically
and by sections Cl. cespitosa in a completely retracted state,
and can find no trace of such a condition, an observation which
confirms my belief that this appearance is due to partial contrac-
tion, owing to the use of alcohol. There is no true cœnosarc
such as occurs in Stylophora; just as there is no true cœnen-
chyme, the calyces being free outwardly from the rest of the
colony. In luxuriant growth and budding, however, according
to Heider, both skeletons and soft tissues of adjacent polyps
may fuse; an observation interesting as probably indicating
the history of the formation of the cœnosarc and cœnenchyme
which characterise many other forms. There is one correction
to be made in his work, which for the sake of future workers
in this field ought to be mentioned here; namely, that in his
Pl. III. he frequently figures as endodermal cells small spherical
bodies with a well-staining nucleus, which are zooxanthellæ or
symbiotic unicellular Algæ, living free in the cœlenteron in

such numbers as often to completely obscure the true endo-
derm, with which they of course have no connection. While
accepting v. Koch's theory as to the origin of the theca from
fusion of the septa, he differs from it in some details, regarding
the "sutures" as merely cracks artificially produced in the
corallum. Septa and tentacles both entocœlic and exocœlic;
mesenteries and their muscles arranged as in Actinia; for
further details, which are very thoroughly worked out, his
paper should be consulted. One point of importance deserves
mention; between the corallum and the structureless meso-
derm-lamella which overlies it immediately and was generally
understood to secrete it, v. Heider detected certain cells, for
the most part scattered, but in some places forming a definite
layer. To these he gave the name calycoblasts, and assigned
the function of coral-secretion; with great justice, as later
researches proved, though their origin was a matter of doubt
till cleared up by v. Koch.

The latter, in a paper on the development of **Astroides
calicularis** (9), brought into notice the following facts.
When first fixed, and before the secretion of the skeleton has
commenced, the embryo is plano-convex, and its ectoderm may
be divided into two regions, corresponding to its surfaces, the
plane disc of attachment, or basal ectoderm, and the convex
portion or lateral ectoderm, the centre of which is invaginated
as the stomodæum. The skeleton first appears as small pellets
of calcium carbonate lying between the basal ectoderm
and the foreign body to which the embryo is
attached, and is therefore outside the animal, and conse-
quently the result of secretion by the ectoderm.
As the corallum is always described in text-books as a product
of the mesoderm, this observation cannot be too strongly
insisted upon. These pellets become, first a ring-shaped disc,
then a complete disc lying between the basal ectoderm and the
foreign body to which the embryo is attached. Where septa
are to be formed the three body layers, endoderm, mesoderm-
lamella, and basal ectoderm, rise upwards as a fold into the
cœlenteron; and as they rise, coral is deposited beneath them

which fuses with the original disc ; the septa are thus also deposited outside the basal ectoderm. They then begin to bifurcate at their distal ends. The originally basal ectoderm to which the secretion of the skeleton is attributable, persists in the adult as the calycoblasts of v. Heider.

Von Koch further asserts that the theca results from the fusion of the bifurcating ends of the septa ; but, though not venturing to deny this, I would point out that he neither describes the process nor gives figures to illustrate it ; whereas, on the other hand, we have the direct evidence of L. Duthiers to the effect that the theca and septa arise independently of each other (" les septa et la muraille ne sont pas unis "), and a figure which appears to bear out his statement. It must, however, be borne in mind that Lacaze Duthiers may have described as theca what v. Koch terms epitheca, a secretion of the lower portion of the lateral endoderm of the embryo which fuses with the periphery of the original basal disc, and ultimately combines also with what he terms the true theca formed as above mentioned, to become the outer wall of the corallum. Were this the case, however, the costæ could not be, as he regards them, the peripheral ends of the septa. But the question can only be finally settled by a study of the embryonic development of widely different forms.

Professor Moseley (**10**) has published a preliminary note on **Seriatopora** and **Pocillopora**. These forms were originally classed with the Tabulata, but his account of their anatomy brings them into close connection with the other Madreporaria at present described. The polyps of Seriatopora are oval in outline, with twelve short tentacles, which in complete retraction are covered over by the indrawn margins of the disc, a condition common in Actiniaria, but very rare in Madreporaria. There are twelve mesenteries, only two of which, the same two in every polyp, are enormously long and bear mesenterial filaments and generative organs. The elongation of this pair of mesenteries deep into the colony suggests an inevitable comparison with the Alcyonaria ; and the similarity is strengthened by the marked orientation of the polyp, for a division into " dorsal "

and "ventral" halves is clearly distinguishable in both soft tissues and corallum. Two of the septa are very rudimentary, and both this fact and the absence of mesenterial filaments on ten of the mesenteries would seem to indicate a degeneration, of which I hope to bring forward a second instance in a future paper. Between the polyps runs a similar canal system to that already described by v. Koch in Stylophora. The anatomy of Pocillopora, so far as mentioned, appears to agree in all respects with that of Seriatopora, and the polyps exhibit the same marked orientation.

Moseley (11) has also described the macroscopic anatomy of three other Madreporarian polyps. His observations on Flabellum are mostly incorporated with my own below, and need not therefore be recapitulated here; and of Stephanophyllia I hope to give a detailed description in a future paper.

Of Bathyactis, which is planoconvex in shape, the plane being the basal surface, he records that on decalcification a lamina of ectoderm and mesoderm separates off from the base. This fact, together with its shape, suggests that the original basal ectoderm of the embryo persists in this species throughout life, in its primitive position, except for such part as grows up with the skeleton (the calycoblasts).

To sum up the undoubted facts elucidated by these observers:

1. The adult Madreporarian polyp is built distinctly on the Actinian type, except for the absence of an external bodywall in some cases (Caryophyllia, Cladocora), which is then replaced physiologically by the imperforate theca.

2. The corallum is a product of the ectoderm, and deposited outside the embryo.

3. This ectoderm persists in the adult as the layer of calycoblasts, to which the continual growth of the corallum is attributable; thus the skeleton is morphologically external to the polyp throughout life.

4. Between this layer and the cavity of the cœlenteron, and clothing every part of the skeleton, is a layer of mesoderm and endoderm, forming the internal body wall.

5. Septa, when present, always lie between a pair of mesenteries (entosepta), sometimes also in the spaces intermediate between pairs of mesenteries (exosepta).

6. Tentacles may be exocœlic as well as entocœlic, but exosepta may be present without corresponding tentacles.

The present classification of the Madreporaria is admittedly unscientific. I have therefore laid stress on what may perhaps seem the trivial point of the relations of septa and tentacles to the mesenterial spaces, as it is probable that, since the morphological differences of the whole group of Zoantharia hexacoralla are very slight, such structural variations might be useful for a new classification; which, if based upon the relations of polyp to skeleton, will be on a far sounder foundation than the present one, which rests upon the skeleton alone.

Flabellum patagonichum (Moseley).

This is an imperforate Madreporarian, belonging to the family Turbinolidæ. As Moseley (11) has given a full descrip- of the specific characters of the corallum in his "Challenger" Report (to which reference should be made for figures of the complete calyx), only a few of them will be mentioned here.

i. The corallum is solitary and conical, the apex of the cone forming a pedicle by which the polyp is attached when young; in the adult the pedicle becomes obliterated, and the coral free (vide figs. 2, 3, Pe.). The outline of the mouth of the calyx is oval (fig. 1). There are four orders of septa, all of which are entocœlic; six of the first order, which meet in an elementary form of columella; six of the second, which are nearly as long as the primary septa; twelve of the third, and twenty-four of the fourth order. In some specimens the full number is not developed. The corallum is about 2 cm. high in a well-grown specimen; and the longer axis of the calyx mouth about 2½ cm., the shorter axis 2 cm. in length.

Along the lines which correspond on the exterior surface of the theca with the attachments of the septa on the interior, are shallow but distinct grooves running from lip of calyx to tip of

pedicle, each corresponding exactly in position with a septum. These do not agree with von Koch's views as to the origin of the theca from fusion of the septa; to accord with which costæ should be developed in this position, such as occur in many forms.

The whole of the exterior surface of the theca shows well-marked lines of growth (fig. 4), so arranged as to appear to indicate that the chief centres of activity for the secretion of coral lie in the septa. Hence the lip of the calyx is slightly dentate (figs. 3, 4).

While the upper fourth of the external surface of the theca is, like the whole of the interior of the calyx, glistening, white and hard, the lower three fourths are soft in texture and brownish. This latter portion was described by Moseley as a "light-brown epitheca." But on decalcification the brown substance falls off as soft flakes, which, by means of sections, are found to consist of dead tissues and algal (?) parasites. There is really no epitheca present, recognisable as such in the adult.

The columella (fig. 3, *col.*) is incomplete, the septa not always meeting regularly along their free edges.

In the retracted condition of the polyp there is no tissue external to the corallum (figs. 1, 2), nothing corresponding to the condition described by Heider in Cladocora and by Koch in Caryophyllia. When expanded, however, the soft tissues almost certainly stretch outwards and downwards over the upper fourth of the exterior of the theca, which is thus kept white and hard, as mentioned above. Were the polyp thus completely expanded to be plunged into a killing fluid, the same appearances would ensue as the above-named observers have described.

ii. **Anatomy.**—This agrees in all essential details with the Actinian type, except in the absence of an external body wall, the whole polyp being enclosed in the corallum (figs. 1, 2). Moseley mentions that in some specimens tissues external to the theca were observed round the lip, and figures them (**11**), pl. xvi, fig. 10, as consisting of ectoderm and mesoderm, but had not the means of studying them by sections. None of my

specimens had any trace of such, and from observations on
Desmophyllum, a closely allied form, I imagine that these
tissues were simply due to the expansion of the polyp, and
contained a continuation of the cœlenteron such as was
described by v. Heider in Cladocora. On decalcification the
polyp appears conical, and divided into a series of wedges by
the spaces where the septa had been. At the base of the
polyp, *i.e.* the apex of the cone, these wedges appear to be
connected together by little bridges of tissue; these latter are
of no morphological importance, being due apparently merely
to the incompleteness of the columella, and their arrangement
varies in different specimens. The polyp consists of a mouth-
disc, bearing tentacles: a stomodæum, which opens into the
cœlenteron, the latter being periaxially divided into exocœles
and entocœles by the mesenteries.

The mouth-disc (fig. 2, MD) is peripherally fastened to
the extreme edge of the lip of the calyx, and is centrally
invaginated into the typical Anthozoan stomodæum.

On the disc are borne the tentacles, which are simple
hollow evaginations of the entocœles, *i.e.* one is placed over
each septum. They are covered by small prominences, each
of which is a " battery " of nematocysts. I have not been able
to determine whether they possess an opening at the tip or not.
They vary in size and position according to the order to which
they belong, the primary tentacles being the largest and the
nearest to the mouth. (*Vide* Moseley (11), pl. xvi, fig. 12.)

The mouth is oval in outline, and at each end of its long
axis is in most cases a well-marked gonidial groove.

Through the periphery of the mouth-disc protrude the
acontia. I have by a fortunate section been able to satisfy
myself that they are ejected through definite openings, not by
rupture of the disc; these are therefore directly comparable to
the cinclides of Actiniæ.

A mesentery of the first order is drawn in fig. 5 to show
the general trend of the muscles, though they are much more
numerous than there represented. They are best seen by
mounting the mesentery whole in glycerine.

In the arrangement of the longitudinal muscles on the inner (entocœlic) faces of the mesentery Flabellum agrees with Actinia; these are the retractors of the polyp. On the outer (exocœlic) faces are ranged the protractors, oblique in direction; these differ slightly in the species, being confined in Fl. alabastrum to the upper third of the mesentery, while the longitudinal fibres extend for its whole length. Both sets of fibres are continued into the tentacles; the oblique muscles of the mesentery becoming their external longitudinal coat, the longitudinal muscles of the mesentery passing into the internal and approximating circular fibres of the tentacle. This apparent change of direction will be understood by fig. 5.

The two pairs of " directive mesenteries " at the ends of the longer axis of the mouth appear to possess the same general direction of the muscle-fibres, though bearing them on reverse faces; but the oblique protractor muscles (in this case entocœlic) are, proportionately to the retractors, somewhat more strongly developed, implying perhaps that the expansion of the polyp is their especial function.

There are no perforations through the mesenteries, such as are described in Actiniæ, putting the chambers in communication.

Both the primary and secondary orders of mesenteries are attached to the stomodæum for its whole length; the tertiaries are attached to the mouth-disc, but, as the latter passes imperceptibly into the stomodæum, no importance is to be attached to this.

What Moseley (11) has termed " the contorted mesenterial filaments," a mass of coils lying on the side of the mesenteries, appear to me after careful investigation to be, in part at least, organs corresponding to the acontia of Actiniæ, namely, long lamellar offsets of the free edge of the mesentery, with one edge thickened to correspond to the mesenterial filament, and charged with very large nematocysts. They protrude in some instances, as above stated, through definite openings in the mouth-disc. Their exact origin from, and relation to, the mesenteries I have not been able to detect owing to the brittle

condition of the specimens, which did not allow of their being dissected out.

The ova are developed on all three orders of mesenteries; as their origin and position does not appear to differ from the type described by the brothers Hertwig for Actinia, no figures are given. I have not seen the testes, hence Flabellum may be regarded as diœcious. The filament is present along the whole course of the free edge of the mesentery, including that region in which ova are developed. The latter is mostly below the part which is characterised by great contortion of the free edge and by (?) the giving off of acontia.

iii. Histology.—The ectoderm of the mouth-disc (fig. 6) is characterised by deeply-staining, very numerous nuclei; and has distinctly the appearance of a secreting layer. It probably produces a similar secretion to the slime poured forth in quantities by an irritated Actinia.

This figure (which is a section along the line a, fig. 2) is taken from a well-grown polyp, and shows traces of the originally basal ectoderm which secretes the corallum (the calycoblasts of v. Heider) ($ch.$, fig. 6). In a younger and actively growing polyp these are much more definitely marked ($ch.$, fig. 7). The nuclei lie in a gelatinous-looking matrix, which stains slightly with borax carmine, but in which no cell outlines are distinguishable. In the calycoblast layer surrounding the septum, at the same height and in the same polyp, the nuclei are much rarer ($ch.$, fig. 8).

The characters of the ectoderm alter considerably on the tentacles; as above mentioned, it is on them raised into a series of knobs, each of which is a "battery" of nematocysts. A transverse section through the wall of a tentacle is shown in fig. 9, and exhibits the structure of a battery; the nematocysts are confined to the peripheral part, and behind them lie a very large number of nuclei, probably instrumental, as was first suggested by v. Heider, in the formation of the cells which replace the ejected nematocysts. On the peripheral face of the mesoderm-lamella lie longitudinal muscle-fibres

continuous with the transverse fibres of the mesentery; on the central face oblique fibres.

The stomodæal ectoderm is not essentially different from that of the mouth-disc; and, though there are well-marked gonidial grooves (food grooves, Mundwinkelfurchen), they show no differentiation of ectoderm comparable to that of Alcyonarians (the "siphonoglyphe" of Hickson).

The whole of the cœlenteron is lined by endoderm of cubical or columnar cells; generally it is only one cell deep, and in the living animal presumably ciliated throughout. At the point where it passes into the thickening known as the mesenterial filament (if that be indeed endodermal in origin) its characters change, and the number of nuclei increases enormously, together with the length of the cells. Its histological appearance entirely bears out what physiological investigation has also shown for the similar filament in Actiniæ, that it is secretory in character, producing a proteolytic fluid (fig. 10).

Nematocysts do not occur apparently in the true mesenterial filament, but only on that portion of it which is continued on to the contorted lamellæ, which I regard, in part at least, as equivalent to the acontia of Actiniæ. Those occurring on the tentacles are of a different size and shape from those which characterise the acontial filament, though in the latter both forms are found. The smaller, occurring on the tentacles, is ·06 mm. × ·01 mm.; the larger, which is only to be found on the acontial filament, is ·1 mm. × ·025 mm. The thread of the latter form is covered with minute barbs, which give it, when coiled up in the capsule, a granular appearance.

Rhodopsammia Parallela (Semper.)

This form, belonging to the family Eupsammidæ, affords a very good example of a perforate Madreporarian. Budding sparsely, it forms no cœnenchyme, so that the polyp can be studied easily and without the complications incident to cœnenchymatous species.

i. Of the **Corallum** the systematic characters have been

already described by Semper (**12**), but certain corrections are to be made in his account relative to the arrangements of the septa. Beautiful figures of the colony will be found in his paper, which contains much valuable and curious information about the group Madreporaria.

The corallum of a polyp is about 30 cm. in height; the calyx, which is as usual oval in outline, measures about 18 mm. in the longer axis, and 9—13 mm. in the shorter. Fresh polyps may be budded off from the side, or, more rarely, from the calyx.

The theca has the porous appearance characteristic of the Perforata, and is marked on the external surface by distinct spinous costæ, or ridges; each of which corresponds externally to the attachment of a septum on the interior surface of the theca (fig. 14).

Both exosepta and entosepta occur in this form. Of true, *i.e.* entocœlic septa, there are only three orders, with occasional traces of a fourth; from the sides of each primary and secondary entoseptum grows out an exoseptum (fig. 14), and the relations of these two classes to each other are rather complicated. Such a system as *a—a* in fig. 22, shows, in a transverse section taken high up in the polyp, the arrangement diagrammatised in fig. 19, consisting of five true entosepta (each of which lies between a pair of mesenteries), and four exosepta alternating with them. In a lower section (fig. 20), the two exosepta which grow out from the sides of adjacent primary and secondary entosepta, fuse over and with the intermediate tertiary septum into one. Lower yet (fig. 21), the two compound septa thus produced in each system meet over and with the secondary septum; so that the columella is due to the irregular fusion (fig. 15) of twelve primary entosepta, distinct for their whole length, and twelve other septa thus elaborately compounded.

ii. **Anatomy.**—In Rhodopsammia, which, like all the other forms as yet described, bears a close resemblance to an Actinia, the mouth-disc, unlike the case in Flabellum, passes into a distinct external body wall of ectoderm, mesoderm, and endo-

derm (extending in some specimens very much further down than
is represented in the diagram, fig. 13). Between this and the
theca lies a narrow space, in which run, parallel to the long
axis of the corallum, lamellæ of tissue, connected on the one
hand with this external body wall, on the other with the tissues
clothing the exterior surface of the theca (figs. 13, 14, 17, M.′)
These lamellæ correspond externally to the attachments of the
mesenteries on the interior surface of the theca, and are appa-
rently continuous with them over the lip of the calyx (fig. 13).
They thus divide the space between body-wall and theca into a
series of long chambers, corresponding to the exocœles and ento-
cœles, in each of which lies a costa. Between these chambers
and the exocœles and entocœles, a system of ramifying canals
permeates the theca, placing the two sets of cavities in com-
munication with one another. The columella is perforated by
a similar system of canals which unites the whole circle of
entocœles and exocœles; there is thus free communication
throughout the whole of the polyp, despite the comparative
preponderance of skeleton over soft tissue. The canals are
composed of endoderm and mesoderm, continuous with the
same layers that clothe all the rest of the skeleton; and in the
meshes of the network lies the corallum, theca, or columella.

The polyp thus consists of an external body wall, mouth-
disc with tentacles, stomodæum, and mesenteries; with a
cœlenteron divisible into columellar canal-system, exocœles,
entocœles, thecal canal-system, and chambers exterior to the
theca, corresponding to, and continuous over the lip with, the
mesenterial chambers.

The body wall and mouth-disc are composed of simple ecto-
derm, endoderm, and mesoderm, agreeing with those of other
Hexactiniæ.

The outline of the stomodæum is oval as usual, but I have
not observed any trace of gonidial grooves at the ends of the
longer axis.

The tentacles, which are simple evaginations, appear to be
entocœlic only; they are so invaginated into pockets on each
side of the septum that it is impossible to make out their

exact size and shape.   This condition is probably due merely to alcoholic contraction, and does not imply that involution is the normal method of tentacular contraction.   A similar invagination had taken place at the bases of the tentacles of Flabellum.   They are covered with nematocysts, which are not so sharply defined into batteries as was the case in Flabellum.

At a varying depth below the lip of the calyx (but generally at a lower point than is represented in fig. 13, which is considerably shortened in the longer axis) the external body wall perishes, owing probably to the various parasites that infest the external surface of most coral thecæ and polyps; notably a sponge, which in some places eats its way right into the theca. The cavity marked f in fig. 15 is thus filled with sponge spicules.   Below the point at which the body wall ends, is visible in some places a thin line of tissue indicated in fig. 15, g, which may or may not be a part of the polyp.   The appearance of the periphery of the theca in such a section suggests very strongly that a secondary line of corallum has been deposited round the circumference to protect the canals from direct communication with the sea water and against the parasites. At the top of fig. 15 the semicircular outline of the canals seems to indicate such a formation.

The mesenteries vary in number, and are, like the entosepta, generally of three orders.   They are divisible into " pairs " as in the other forms described, and possess the same arrangement of longitudinal retractor muscles on their entocœlic faces, with the usual difference in the two directive pairs.   The trend of these muscles is roughly indicated in fig. 13 ; but their minuteness renders it impossible to recognise the arrangement of the protractor muscles, though they are just visible in microscopic sections.   There appears to be but little contortion of the free edge of the mesentery ; and the traces of any organs resembling acontia are rare.   This, however, may be due to deficiency of material, which has much hampered my investigation of this form.

Both primary and secondary mesenteries appear to be united to the stomodæum for its whole length ; those of the third

order become disconnected very high up, and do not run deep down into the colony, the cavities in which they lie disappearing among the other perforations of the theca.

The number of pairs of mesenteries right and left of the " directives " is not necessarily equal.   Complete systems, both of mesenteries and septa (1, 3, 2, 3, 1, in notation), are generally found only at the ends of the long axis of the calyx, *i.e.* in the neighbourhood of the directives.   This has been noticed in many other corals.

That the almost exact correspondence of costæ with septa, and of the external lamellæ (M′ in the figures) with the mesenteries, adds to the probability of the correctness of v. Koch's view, is undeniable.   But it is to be noted, that no muscles are to be recognised on the mesoderm plates of these lamellæ, as would probably be the case had they once been part of the mesenteries ; nor in the highest sections of the decalcified polyp are any cases of decaying tissue visible, where the growing theca is supposed to have cut them.

iii. Histology.—This is of such a simple character as to hardly require comment.   The ectoderm is composed of simple columnar cells, the endoderm of similar but more cubical cells. Calycoblasts are present, but in small numbers in comparison with Flabellum.   Nematocysts are of two forms and sizes, of which, as in Flabellum, the smaller is the only one occurring on the tentacles.   Of the mesenterial filament, as unusual in outline, a sketch is given in fig. 16.

In conclusion, I have to acknowledge my obligations to Professor Moseley for much kind assistance and most of my material ; to Professor Milnes Marshall for valuable advice ; to Mr. John Murray, of the " Challenger " office, for several specimens of Flabellum ; and lastly, to the anonymous donor of the Berkeley Fellowship, whose generosity has enabled me to pursue the investigation.

CLASSIFICATION OF THE ZOANTHARIA (HEXACORALLA).

1. Actiniaria (Malacodermata).
    i. Hexactiniæ .   .   .   **Actinia.**
    ii. Edwardsiæ.
    iii. Zoantheæ.
    iv. Ceriantheæ.
2. Madreporaria (Sclerodermata).
    A. Imperforata (Aporosa).
        i. Turbinolidæ   .   **Flabellum.**
                           Caryophyllia.
        ii. Oculinidæ .   .   **Stylophora.**
        iii. Pocilloporidæ   .   **Pocillopora.**
                           Seriatopora.
        iv. Astreidæ .   .   **Cladocora.**
    B. Fungida.
        i. Fungidæ .   .   **Bathyactis.**
    C. Perforata.
        i. Eupsammidæ .   **Stephanophyllia.**
                           Rhodopsammia.

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DESCRIPTION OF PLATES XL, XLI, and XLII,

Illustrating Mr. G. Herbert Fowler's Paper on "The Anatomy of the Madreporaria."

b. w. Cut edge of internal body-wall. *ch.* Calycoblast layer. *Cœl.* Cœlenteron. *C.* or *Col.* Columella. *Cos.* Costa. *D.* "Directive" septum and mesenteries. *Ect.* Ectoderm. *En.* Endoderm. *En. S.* Entoseptum. *Ex. S.* Exoseptum. *M.* or *Mes.* Mesentery. *M'.* "Peripheral" part of mesentery. *M. D.* Mouth-disc. *Me.* Mesoderm. *Me'.* Mesenterial muscles. *M. F.* Mesenterial filament. *m. long.* Longitudinal muscles. *m. obl.* Oblique muscles. *n.* Nematocyst. *Pe.* Pedicle. *S.* Septum. *St.* Stonodæum. *Te.* Tentacle. *Th.* Theca.

Fig. 1.—Section through two quarters of Flabellum, diagrammatic, the right alf showing the primary and secondary mesenteries attached to the stomodæum, taken along the line *b*, Fig. 2, the left being lower down in the polyp, where the mesenteries have all developed filaments, taken along the line *c*, Fig. 2. *A.* Exocœle. *B.* Entocœle. i, ii, iii. Orders of septa and mesenteries. Corallum coloured deep black throughout the figures.

Fig. 2.—Diagrammatic section along the line *a*, Fig. 1, *i. e.* in an exocœle, so that the external face of the mesentery is seen flat, while the mouth-disc and internal body-wall are cut. The contortions of the free edge of the mesentery are omitted.

Fig. 3.—View of half of the corallum of Flabellum, showing the relations of pedicle, theca, and septa, and the incomplete union of the septa marked *x*, in Fig. 1 into a columella.

Fig. 4.—Portion of the lip of the calyx of Flabellum, viewed from the exterior by transmitted light, to show the grooves, i, ii, iii, corresponding to the septa of those orders, with the lines of growth of the theca curving upwards at those points.

Fig. 5.—Primary mesentery and base of primary tentacle of Flabellum, showing the direction of the muscles, contortions of the free edge omitted.

Fig. 6.—Section along the line *a*, Fig. 2, from a full-grown specimen, with the layer of calycoblasts between mesoderm and theca.

Fig. 7.—Similar section through the internal body-wall of a younger polyp, in which the calycoblasts are much better marked.

Fig. 8.—Section through the tissues clothing the septum of a young Flabellum.

Fig. 9.—Section through the wall of a tentacle, including one complete "battery."

Fig. 10.—Section through a mesenterial filament of Flabellum.

Fig. 11.—Transverse section through part of a mesentery, to show the mesodermal pleatings on which lie the muscles.

Fig. 12.—Transverse section of an acontium of Flabellum.

 Fig. A. Transverse diagrammatic section of Caryophyllia (after v. Koch). a. Septa. b. Mesenteries.

 Fig. B. Similar section through Caryophyllia, in a lower plane than A (after v. Koch). a. Septa. b'. Central. b''. Peripheral parts of the mesentery. c. Costæ. th. Theca.

Fig. 13.—Diagram of a longitudinal section of Rhodopsammia, considerably shortened in the longer axis; the right half of the figure taken along the lines c. c. in Figs. 14 and 15, i.e. in an exocœle; the left along the lines d. d. in the same figures, and therefore cutting through a septum and a tentacle. c. Cut edge of external body-wall. d. Cut edge of tissues clothing theca and columella. B. Tissue clothing the entoseptum, which is seen projecting from behind the mesentery. On the left side the inner face of a mesentery (M) is seen similarly projecting from behind the septum. The costæ being in this form rows of spines, appear as projections in both transverse (Fig. 14) a longitudinal (Fig. 13) sections.

Fig. 14.—Transverse section of half of the calyx of Rhodopsammia, along the plane a, Fig. 13 (camera drawing). The numerals 1, 2, 3 are placed in the entocœles formed by a pair of mesenteries of those orders. Complete systems, 1, 3, 2, 3, 1, are only found in the region of the directives. The dashed numerals, 1', 2', 3', are placed in the external chambers which correspond to the entocœles. ext. b. w. External body-wall. Corallum deep black, soft tissues in lighter black lines.

Fig. 15.—Similar section along the plane b, Fig. 13. The septa have fused into the columella, and are numbered 1, 2, 3, according to their orders. f. Cavity filled with sponge spicules. g. Line of tissue which may belong to the polyp.

Fig. 16.—Mesenterial filament of Rhodopsammia in transverse section.

Fig. 17.—Part of Fig. 14, enlarged to show the relations of the three body-layers. Mesoderm black, corallum grey. c. Thecal canal system in transverse section. c'. External chambers, corresponding to exocœles and entocœles, in each of which lies a costa.

Fig. 18.—Part of the thecal canal system of Rhodopsammia, after removal of the corallum by decalcification.

Figs. 19, 20, and 21.—Diagrams of the relations of a complete system of septa of Rhodopsammia at different heights. The numerals are placed at the bases of the entosepta.
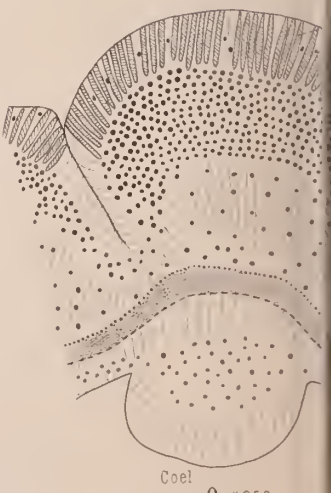
Fig. 22.—Calyx of Rhodopsammia, viewed from above. From a specimen in the British Museum.
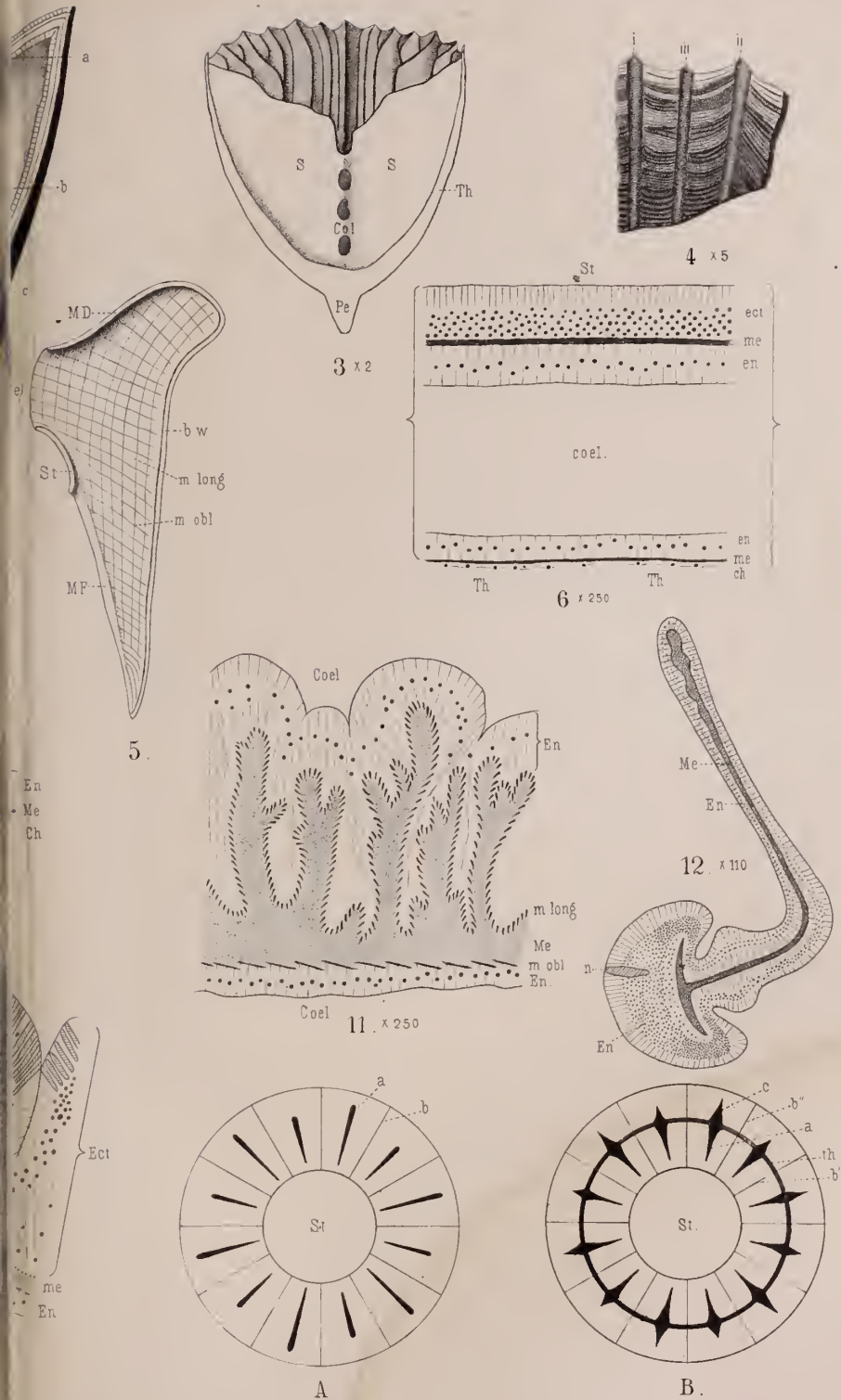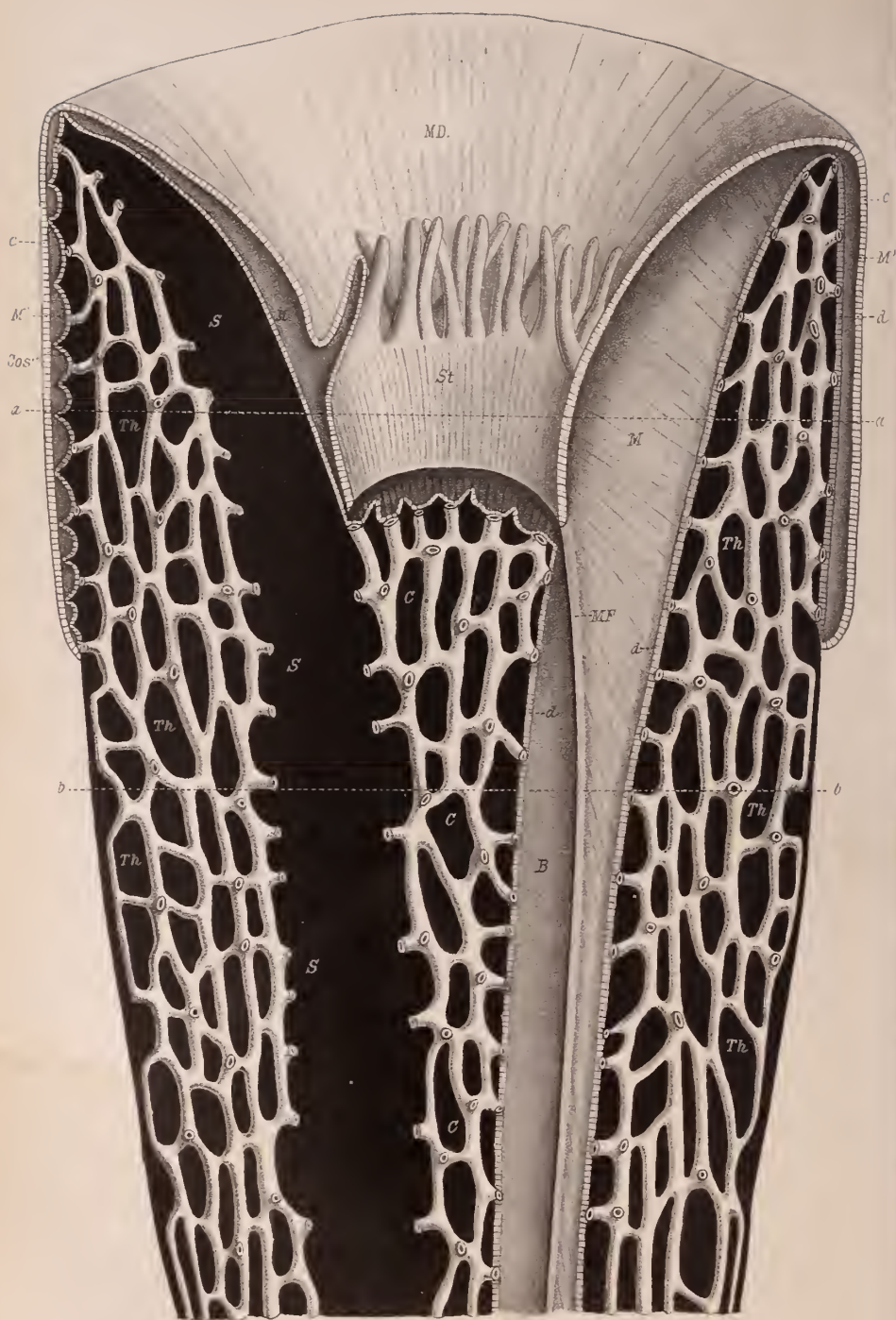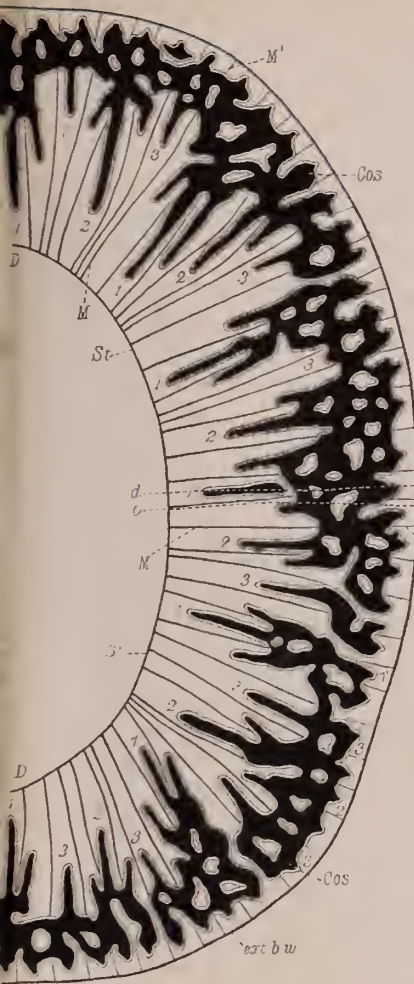
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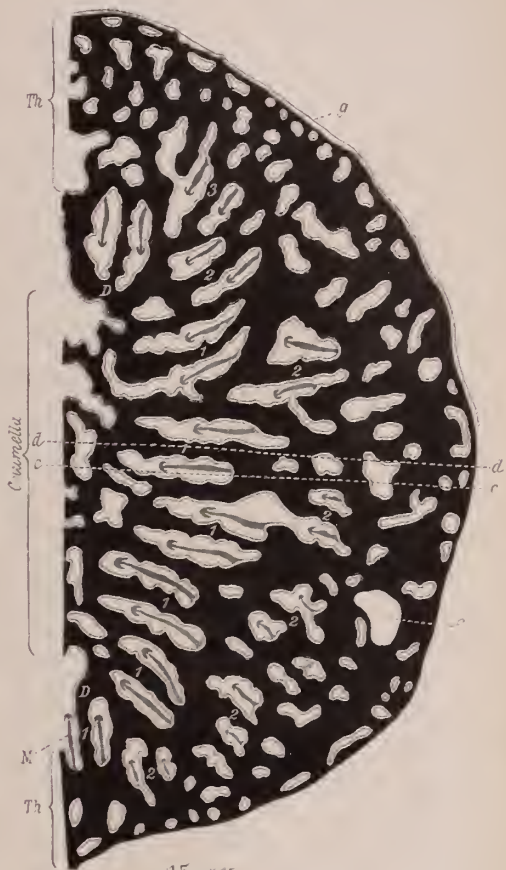
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17 ×87

18. × 100.

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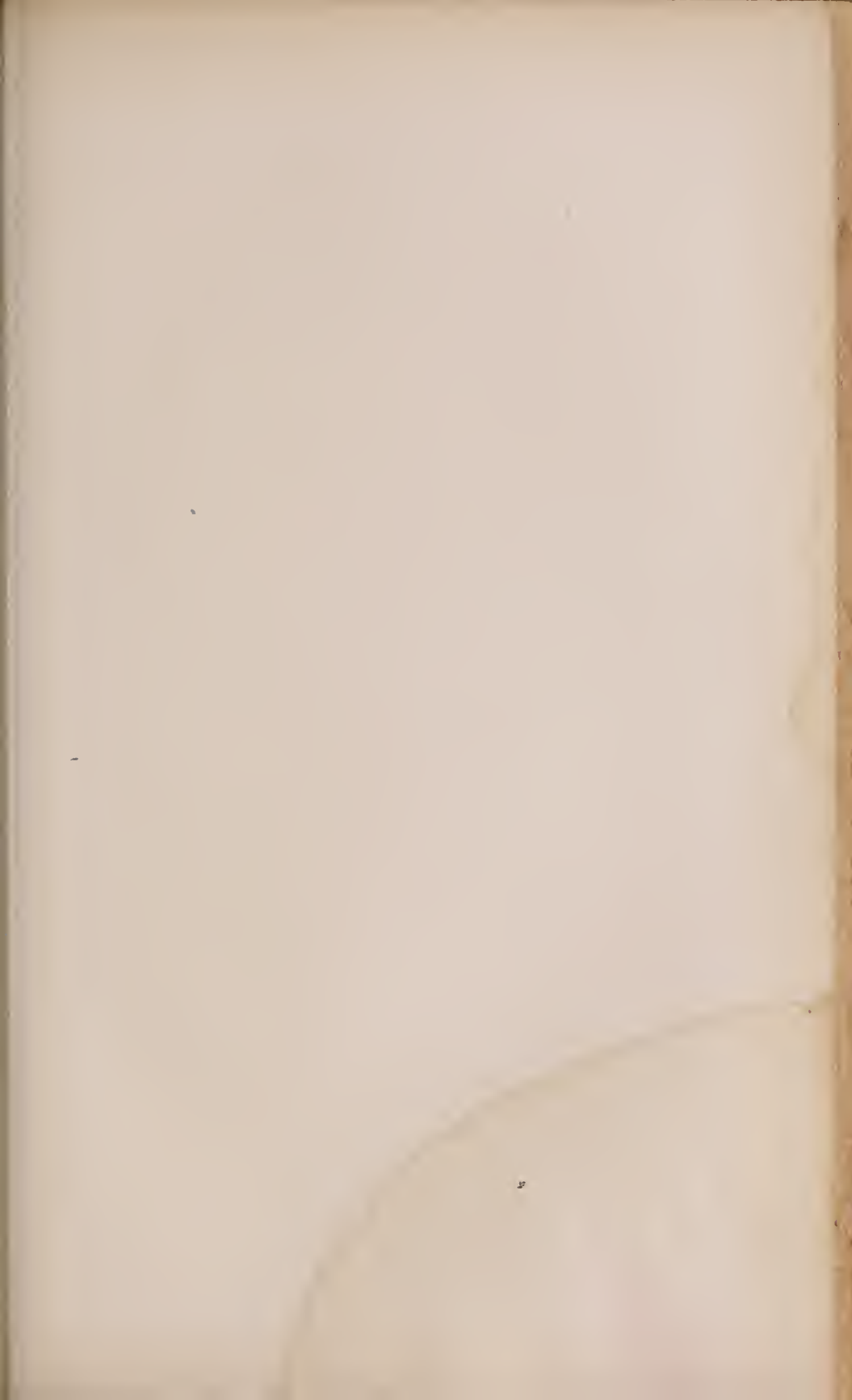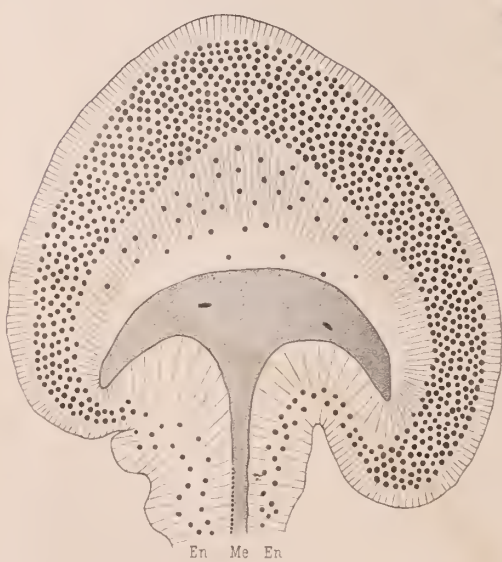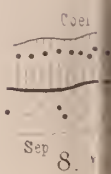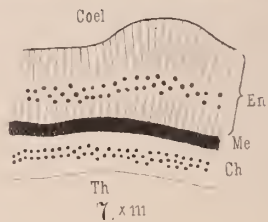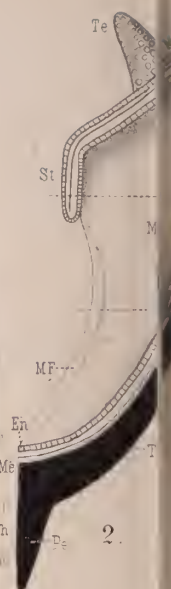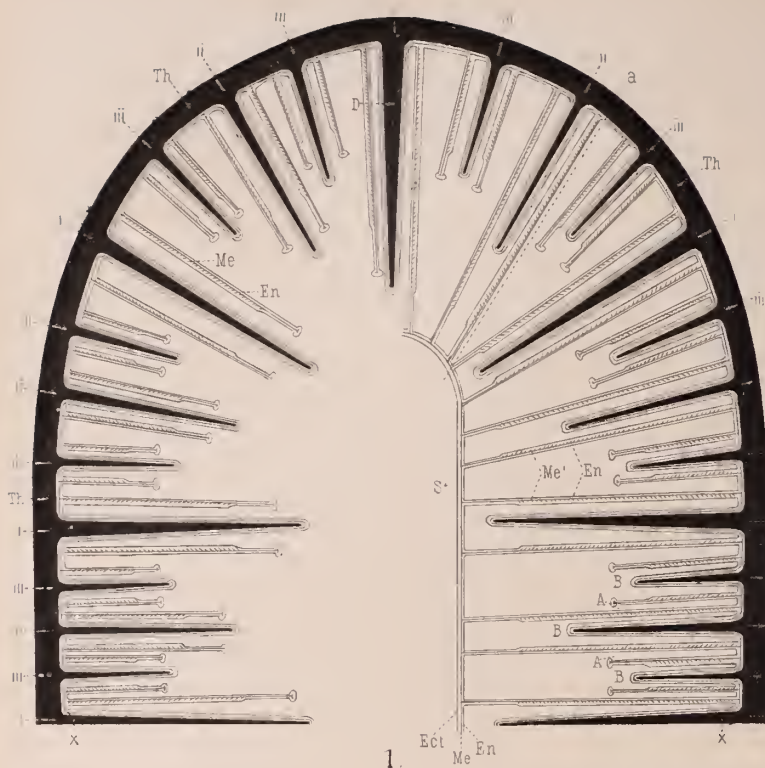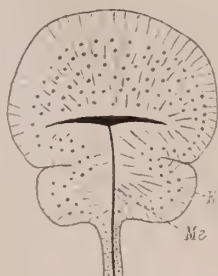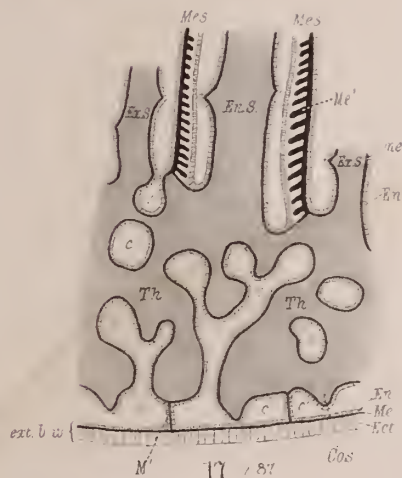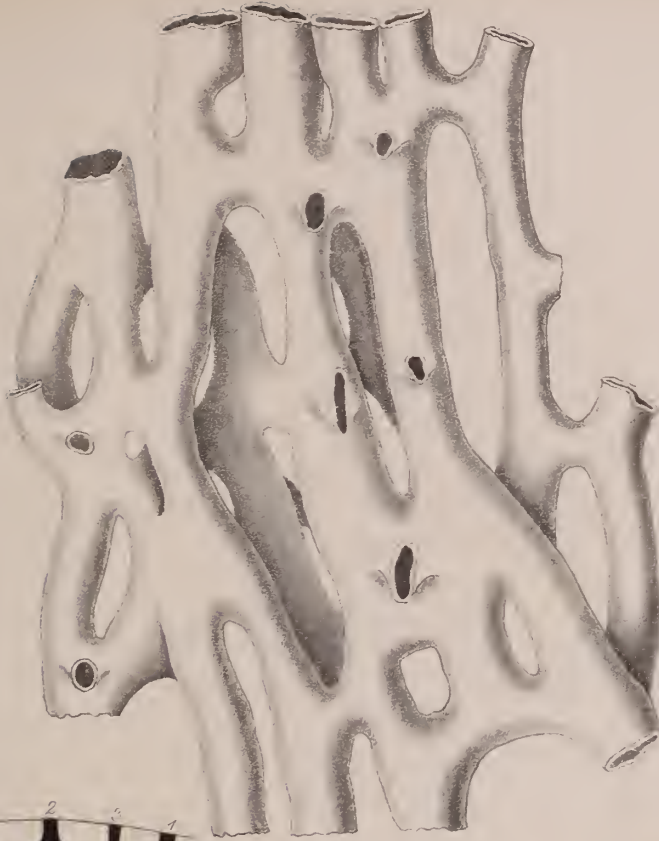
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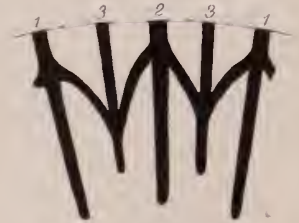
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INDEX TO JOURNAL.

VOL. XXV, NEW SERIES.